DISSERTATION

DETERMINANTS OF GEOGRAPHIC DISTRIBUTION IN WESTERN NORTH AMERICAN MONKEYFLOWERS

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ABSTRACT

DETERMINANTS OF GEOGRAPHIC DISTRIBUTION IN WESTERN NORTH AMERICAN MONKEYFLOWERS

The geographic range of a species represents the basic unit of biogeography. Despite ample evidence that properties of geographic ranges vary among species, we do not fully understand the ecological and evolutionary processes underlying these patterns, thereby hindering our ability to forecast changes in species' distributions in response to changing environments. Key hypotheses about variation in geographic range size among species emphasize the roles of ecological niche properties and the connectivity of suitable habitat. In the first study of my dissertation, I combined primary occurrence data with climate variables to test the relative importance of these hypotheses in 72 species of western North American monkeyflower (genus *Mimulus*). Climatic niche breadth, via its effect on the amount of suitable habitat, was a strong predictor of geographic range size, whereas climatic niche position (relative to regional climate) and connectivity of climatically suitable habitat were not.

Given the role of climatic niche breadth in shaping geographic range sizes in *Mimulus*, the goal of the second study of my dissertation was to examine the relationship between thermal tolerance (an important axis of niche breadth) and range size experimentally using 5 pairs of closely related *Mimulus* species with differing range sizes. Within four species pairs, the more geographically widespread species had a broader thermal tolerance than the narrowly distributed species, providing further support for the hypothesis that species with broader niches are able to achieve larger geographic ranges. Further, within each species pair, the species with broader

thermal tolerance encompassed greater variation in temperature across its geographic range and higher genetic variation for thermal tolerance than the species with narrower thermal tolerance, supporting the hypotheses that climatic variability and genetic variation in ecologically important traits can explain variation in environmental tolerance among species.

Although species vary in range size, every species has a limited geographic range, leading to the question of what prevents a species from expanding its range via niche evolution. Thus, in the third study of my dissertation, I tested whether adaptation at geographic range margins is constrained by insufficient evolutionary potential. To do so, I used artificial selection experiments to quantify genetic variation in flowering time for populations from the northern edge, center, and southern edge of the geographic range of the scarlet monkeyflower (*M. cardinalis*). Contrary to prediction, southern populations exhibited significantly greater responses to selection (and thus evolutionary potential) than northern or central populations. Together, these results highlight an important role of niche breadth in explaining variation in geographic range size among species, and reveal variation in evolutionary potential that facilitates niche and range expansion within and among species.

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TABLE OF CONTENTS

1. INTRODUCTION

Most species are rare, and all species occupy a limited number of areas, yet the causes of variation in the sizes and limits of species' geographic distributions are poorly understood. Identifying constraints on species' ranges provides important insights into ecological and evolutionary processes such as dispersal, speciation, extinction, and adaptation. Specifically, species' distributions are contingent on the geography of speciation and extinction (Mayr 1963), and the overlap of species' ranges across geography creates variation in spatial patterns of diversity. Many early works expressed interest in the processes driving variation among species in the size, structure, placement, and limits of geographic ranges (e.g., Hooker 1853, Darwin 1859, Wallace 1876, among many others). In the early 20th century, Willis (1922) compared the geographic distributions of endemic and non-endemic flora in Sri Lanka (then known as "Ceylon"), and formulated the "Age and Area" hypothesis, which predicts that all else being equal, on average, older species should have had more time to disperse, and thus should have larger geographic ranges than younger species. This hypothesis was quickly refuted, largely due to its dismissal of the role of local adaptation and natural selection in shaping species' ranges (Stebbins and Major 1965). What Willis failed to acknowledge is that range expansion occurs not only via dispersal into suitable habitat, but also by means of local adaptation to previously unsuitable habitat at and beyond range margins (Kirkpatrick and Barton 1997).

Thus, fundamental to understanding mechanisms underlying species' ranges is the concept of the ecological niche, which can be defined as the set of environmental conditions across which species can achieve non-negative population growth (Hutchinson 1957, Chase and Leibold 2003). If geographic range and ecological niche boundaries are in equilibrium, then the

edges of geographic ranges should coincide with ecological niche limits (Pulliam 2000). However, there is widespread evidence that species harbor ample genetic variation in ecologically relevant traits (Dudley et al. 1974), and niche evolution can occur rapidly (Schluter 1996). Conversely, niches may be conserved over evolutionary timescales (Peterson et al. 1999, Wiens 2004). Although some species' ranges are not at equilibrium with current environments (Svenning and Skov 2004), many species show reduced fitness and negative population growth beyond current range boundaries (reviewed in Sexton et al. 2009). Concordance between range and niche limits indicates constraints on niche evolution via adaptation to novel conditions beyond the range (Antonovics 1976). In the early 20th century, much effort was dedicated towards associating environmental variables with geographic range limits (Brown et al. 1996). For example, the altitudinal boundaries of the geographic distributions of several desert plant species coincide with winter temperature variables (Shreve 1914). By the later part of the 20th century, the study of geographic ranges and the relationships among niche and range variables received considerable attention (e.g., MacArthur 1972), with much work focusing on latitudinal gradients in range size and niche breadth (e.g., Janzen 1967).

Variation among species in range properties has been extensively documented (Gaston 2003), yet we still do not fully grasp the processes underlying these patterns, thereby hindering our ability to forecast changes in species' distributions in response to changing environments. For my dissertation, I studied the mechanisms underlying two main aspects of species' geographic ranges: 1) the overall size and spatial extent of species' distributions, and 2) the geographic margins of these distributions.

Though every species has a limited geographic distribution, range limitation among species can vary by up to 12 orders of magnitude (Brown et al. 1996). Even closely related

species with similar biogeographical and evolutionary histories can differ dramatically in range size (Darwin 1859, Willis 1922). Several factors have been implicated in driving variation in range size among species, including body size, species age, properties of species' ecological niches, and colonization ability, among others (reviewed in Gaston 2003), but the strength and relative importance of each factor in explaining such variation remains uncertain. Properties of species' ecological niches, defined as the set of environmental conditions under which births exceed deaths, may explain differences in geographic range size among species (Hanski 1982, Brown 1984). The niche breadth hypothesis posits that the range of environmental conditions under which species are able to persist is a determinant of range size (Hanski 1982, Brown 1984), thus predicting a positive relationship between niche breadth and range size. Among species, variation in geographic range size has been shown to be strongly driven by variation in ecological niche breadth, or environmental tolerance (Slatyer et al. 2013), but few studies have assessed the relative importance of the niche breadth and other mechanisms in driving patterns of range size variation among species, and even fewer have experimentally tested this hypothesis.

Independent of the relationship between niche breadth and range size, understanding the mechanisms that drive variation in environmental tolerance among species would further our understanding of inter-specific variation in evolutionary potential and vulnerability to climatic changes. A species can accrue environmental tolerance in a number of ways. First, a species may consist of phenotypically plastic genotypes that maintain high performance across a broad range of environmental conditions (Baker 1965). Second, species may consist of specialized genotypes within each population (Bolnick et al. 2003). Third, locally adapted populations may result in a broad species-level environmental tolerance (Ackerly 2003). Both extrinsic factors, such as climatic variability (Janzen 1967, Stevens 1989), and intrinsic factors, such as fitness tradeoffs

(Huey and Hertz 1984, Futuyma and Moreno 1988) and genetic variation in ecologically important traits (Kellermann et al. 2009) may constrain the evolution of a broad environmental tolerance, potentially leading to variation in environmental tolerance among species.

Although species vary in range size, every species has a limited geographic range (Gaston 2003), leading to the question of what prevents a species from continually expanding its range by broadening its niche. Several classes of hypotheses have been proposed to explain why species may fail to expand their ranges via niche evolution. One hypothesis is that gene flow from large, locally adapted central populations at the range center might introduce maladaptive alleles to populations at range margins (Haldane 1956). Another hypothesis, which is the focus of my dissertation research, is that populations at range margins lack sufficient genetic variation to respond to natural selection, thereby leading to evolutionarily stable range limits (Antonovics 1976). Despite extensive theoretical examination of these hypotheses, empirical tests remain scarce.

Dissertation objectives

To examine the role of niche breadth in driving variation in geographic rang size among species, and to assess whether adaptation at the edges of a species' range is constrained by a lack sufficient genetic variation in ecologically important traits, I conducted three studies using the western North American monkeyflowers (genus "*Mimulus*") as a model system. In the first study of my dissertation, I used primary occurrence data and climatic layers to estimate climatic niche breadth and position (relative to average regional climate), connectivity of climatically suitable habitat, and geographic range size of 72 western North American *Mimulus* species. I then assessed the relative importance of climatic niche properties and connectivity of climatically suitable habitat in explaining variation in the amount and occupancy of climatically suitable

habitat, respectively, and in turn, variation in geographic range size. Recent research highlights the utility of experimental approaches for quantifying dimensions of the niche (Calosi et al. 2008, Calosi et al. 2010). Thus, the second study complements the correlative approach described above by experimentally quantifying niche dimensions for a smaller subset of closely related species that vary in range size, allowing for a mechanistic understanding of how broader niches may lead to larger ranges. I experimentally quantified thermal tolerance for five pairs of closely related *Mimulus* species that differ in geographic range size to test the hypothesis that species that are geographically widespread have broader thermal tolerances than species that are geographically restricted. Further, I examined the roles of quantitative genetic variation, climatic variability, plasticity, and specialist-generalist trade-offs in shaping patterns of thermal tolerance. Results from the second study pointed to quantitative genetic variation as an important mechanism limiting thermal tolerance, but genetic variation in ecologically important traits can vary across species' ranges (Antonovics 1976). Thus, the objective of the third study was to assess whether populations from the northern and southern edges of the geographic range of the scarlet monkeyflower, *Mimulus cardinalis*, had lower adaptive potential than populations from the range center. To do so, I compared responses to artificial selection on flowering time, a key phenotypic trait that likely influences fitness, among populations across the geographic range.

Understanding the factors that shape species' distributions can improve our ability to prioritize species and areas of conservation concern, forecast species' vulnerability to climate change, and predict the rate and spread of invasive species. In the Conclusions and Synthesis section, I discuss the overall significance and implications of my dissertation work.

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2. IDENTIFYING THE PATHS LEADING TO VARIATION IN GEOGRAPHICAL RANGE SIZE IN WESTERN NORTH AMERICAN MONKEYFLOWERS[1]

Summary

Closely related species can vary tremendously in size of geographical range, yet the causes of such variation are poorly understood. Prominent hypotheses about range size emphasize effects of niche properties and habitat connectivity via the amount and occupancy of suitable habitat, respectively. Previous studies have examined single hypotheses in isolation; however, we assessed the relative importance of these effects along with their potential interactions, using western North American monkeyflower species (genus *Mimulus*) as a study system. We used primary occurrence data and climatic layers to estimate climatic niche breadth and position (relative to average regional climate), connectivity of climatically suitable habitat, and geographical range size of 72 monkeyflower species. Using path analysis, we then assessed the relative importance of climatic niche properties and connectivity of climatically suitable habitat in explaining variation in the amount and occupancy of climatically suitable habitat, respectively, and in turn, variation in geographical range size. We documented strong support for the hypothesized effects of climatic niche breadth, but not niche position and connectivity of climatically suitable habitat. Amount of climatically suitable habitat explained more variation in range size than occupancy of climatically suitable habitat, with amount and occupancy of suitable habitat together explaining *c.* 83% of the variation in range size. To our knowledge, this is the first study to show that climatic niche breadth, via its effects on the amount of climatically

[1] Sheth, S.N., Jiménez, I., and A.L. Angert. In press. Identifying the paths leading to variation in geographical range size in western North American monkeyflowers. *Journal of Biogeography*.

suitable habitat, is a strong predictor of geographical range size, thereby improving our understanding of the mechanisms driving species rarity.

Introduction

Geographical range size can vary tremendously among species, yet we still do not fully understand the causes of such extreme variation. The present-day distributions of species are contingent upon the geography of speciation and extinction and are influenced by legacies of geological and climatic history (Mayr, 1963). However, even closely related species with similar biogeographical and evolutionary histories can differ dramatically in range size (Darwin, 1859). Several factors have been implicated in driving variation in range size among species, including body size, species age, properties of ecological niches and species' colonization ability, among others (reviewed in Gaston, 2003), but the strength and relative importance of each factor in explaining such variation remains uncertain. Understanding the processes that shape the distributions of species can provide important insights into ecological and evolutionary processes, such as dispersal, speciation, extinction and niche evolution, while also improving our ability to prioritize species and areas of conservation concern, forecast species' vulnerability to climate change, and predict the rate and spread of invasive species.

Properties of species' ecological niches, defined as the set of environmental conditions under which the intrinsic rate of increase is non-negative (Chase & Leibold, 2003), may explain differences in range size among species through their effects on the amount of suitable habitat, defined as the geographical area (within a study region) over which the intrinsic rate of increase is non-negative. The niche breadth hypothesis posits that species able to attain non-negative population growth rates across a broad range of environmental conditions tend to achieve larger geographical ranges because they have more suitable habitat than species with narrower niches

(Fig. 2.1a; Hanski, 1982; Brown, 1984). Thus this hypothesis predicts a positive effect of niche breadth on range size via the amount of suitable habitat (Fig. 2.2a). Another hypothesis emphasizes niche position, which is the location of a species' niche relative to the central tendency of environmental conditions in a study region. Species with a low niche position occupy environmental space that is non-marginal in the sense that it is near the central tendency of environmental conditions within a study region. In contrast, species with a high niche position occupy environmental space that is marginal because it is far from the central tendency of environmental conditions within a study region (Seagle & McCracken, 1986). We emphasize that the definition of niche position adopted here (Fig. 2.1a) differs from that used in the resource utilization literature (Roughgarden, 1974). The niche position hypothesis proposes that species with a low niche position have more suitable habitat and thus larger range sizes than species with a high niche position (Fig. 2.1a; Hanski *et al.*, 1993). Hence this hypothesis predicts a negative effect of niche position on range size mediated by a negative effect of niche position on the amount of suitable habitat (Fig. 2.2a). Niche breadth and position may be negatively related if species with broad niches generalize on environments that are frequent across the study region, and species with narrow niches specialize on environments that are infrequent in the study region, but such a relationship need not exist (Fig. 2.1b). For example, a species with a narrow niche could specialize on an environment that is abundant in the study region and thus may achieve a large range. If more than one form of rarity is at play (e.g. high niche position and narrow niche breadth), then species may be doubly at risk of extinction.

Variation in species' colonization abilities may also explain differences in geographical range size. The colonization ability hypothesis (Lester *et al.*, 2007) suggests that species with a high colonization ability can become established in more sites and thus achieve larger ranges

than species with a poor colonization ability, predicting a positive relationship between colonization ability and range size across species. Colonization ability is a product of intrinsic factors, such as traits that affect mobility (e.g. morphological traits such as wing size in insects and seed size in plants) and/or establishment (life-history traits such as propagule number), and extrinsic factors, such as attributes of the landscape that facilitate movement (Lester *et al.*, 2007; Gaston, 2009). A species should have a higher extrinsic colonization ability if patches of suitable habitat are well-connected rather than highly fragmented (Lester *et al.*, 2007). High connectivity of suitable habitat should facilitate movement and hence site colonization, thereby allowing species to occupy a larger fraction of available suitable habitat and achieve larger ranges (Fig. 2.2a). Consequently, the colonization ability hypothesis predicts a positive effect of connectivity of species' suitable habitat across the study region on geographical range size, mediated by a positive effect of connectivity of species' suitable habitat on occupancy of suitable habitat (Fig. 2.2a).

Studies have documented a positive relationship between niche breadth and range size across a variety of taxa and spatial scales (Slatyer *et al.*, 2013) but such a relationship may be an artefact of widely distributed species occupying a broader range of environmental conditions by chance (Gaston, 2003; Davies *et al.*, 2009). While many studies have examined the effects of niche properties or habitat connectivity on range size in isolation (e.g. Lester *et al.*, 2007), few have assessed the relative importance of each in explaining variation in range size among species (but see Hurlbert & White, 2007; Laube *et al.*, 2013) and even fewer have scrutinized the mechanistic pathways by which niche properties or habitat connectivity are hypothesized to affect range size. In this study, we assessed the relative importance of the mechanistic pathways proposed by the hypotheses outlined above, along with their potential interactions, in a group of

closely related species of western North American monkeyflower (genus *Mimulus*, renamed *Erythranthe* in Barker *et al.*, 2012), an emerging model system in ecological and evolutionary studies. We combined occurrence records with climatic variables to estimate range size, climatic niche properties and the connectivity, amount and occupancy of climatically suitable habitat for 72 *Mimulus* species. To disentangle potential artefacts resulting from geographically widespread species occupying greater climatic variation than restricted species simply by chance, we tested all the relationships against those derived from a null model that randomized the location of species' geographical ranges across the study region. To our knowledge, this study is the first to show that climatic niche breadth, via its effects on the amount of suitable habitat, is a strong predictor of geographical range size. Specifically, our results suggest that niche breadth is the best predictor of range size in western North American monkeyflowers, providing strong evidence that species with narrow climatic niches have a limited ability to achieve large ranges.

Methods

Study system

The monkeyflower genus *Mimulus* (Phrymaceae) is a diverse group of wildflowers that occurs worldwide, with *c*. 90 of the global total of 120 species occurring in western North America (Beardsley *et al.*, 2004). *Mimulus* species occupy a wide variety of habitats, including aquatic, alpine, grassland and desert environments, can be herbaceous or woody, annual or perennial, and can exhibit complete outcrossing, obligate selfing or exclusively asexual reproduction (Wu *et al.*, 2008). Because the geographical distributions of *Mimulus* species are well described and largely encompassed within protected lands in western North America, and vary markedly in size (Beardsley *et al.*, 2004; see Fig. S1.1 in Appendix 1.1), *Mimulus* represents an ideal group for testing hypotheses regarding the variation in range size among

species (Wu *et al.*, 2008). Furthermore, there is an existing phylogenetic hypothesis for *Mimulus* allowing for phylogenetically controlled studies (Beardsley *et al.*, 2004; Grossenbacher & Whittall, 2011). A recent taxonomic revision of western North American *Mimulus* (Barker *et al.*, 2012) has proposed primarily nomenclature changes but retained the major patterns of the phylogenetic hypothesis used here, and did not alter our main results (see Appendix 1.2).

Species occurrence data

To estimate species' geographical distributions, we compiled locality data from herbarium databases of specimen records and our own collections (see Table S1.1 in Appendix 1.3), resulting in *c.* 17,000 georeferenced occurrences for 82 species of *Mimulus* that occur in western North America. With the exception of excluding disjunct populations of *M. floribundus* in Arkansas (Nesom, 2012), we estimated the species' known global distributions. To augment the sampling of geographical regions and species for which there were few georeferenced records, we used locality descriptions from herbarium specimen labels to georeference an additional *c.* 500 herbarium specimen records. We removed records with large uncertainty in locality data (e.g. conflict between the description of the collecting locality and the geographical coordinates on specimen labels). Of the 82 species with locality data, 10 were known from fewer than three 5-arc minute pixels (see details on spatial resolution below), precluding our ability to estimate niche properties, connectivity and range size, resulting in a final sample size of 72 species (see Fig. S1.2 in Appendix 1.1).

Climatic niche models

We modelled the climatic niche of each species to estimate its climatic niche breadth and position, and the amount and connectivity of climatically suitable habitat. Although reducing the niche to only climatic dimensions ignores potential interspecific differences in edaphic

specialization, it allows a broad-scale comparison of niche properties across a large number of species at a continental scale. We focused on climatic variables that probably affect the survival and reproduction of *Mimulus* species. Of the 19 climatic variables available from the WorldClim database (Hijmans *et al.*, 2005; http://www.worldclim.org/), we selected seven that encompass average and extreme conditions of temperature and precipitation: mean annual temperature, mean diurnal range, temperature annual range, mean temperature of wettest quarter, annual precipitation, precipitation seasonality, and precipitation of warmest quarter. These variables were not highly correlated ($r < 0.75$) among 10,000 points placed randomly across the study region, defined as a minimum convex polygon drawn around western North American *Mimulus* species occurrence points and buffered by *c.* 100 km (Fig. 2.3a). We used climate data at a 5-arc minute resolution (*c.* 10 km × 10 km) and Albers equal area conic projection of North America to obtain equal-area grid cells, which are better suited for range size calculations and ecological niche models (Elith *et al.*, 2011).

To model the climatic niche of each species, we used the maximum entropy algorithm MAXENT 3.3.3k (Phillips *et al.*, 2006), a machine-learning procedure that only requires presence data and performs well compared with other methods, even for relatively small sample sizes characteristic of rare species (Elith *et al.*, 2006; Pearson *et al.*, 2007). For each species we removed duplicate records from each grid cell. We used MAXENT's default values for the 'regularization multiplier' parameter (= 1), the number of maximum iterations (= 500), the convergence threshold (= 0.00001) and feature types ('auto features'). To quantify climatically suitable habitat for each species, we converted MAXENT's output of continuous suitability values into a binary map based on a threshold of the lowest suitability value among known occurrences (lowest presence threshold; Pearson *et al.*, 2007). This threshold defines climatically suitable

grid cells as those that are predicted to be at least as climatically suitable as the lowest suitability value in which a species' presence has been documented, thus eliminating the possibility of omission errors and allowing for the quantification of climatically suitable habitat (Fig. 2.3b). To evaluate model performance for each species known to occur in ≥ 10 pixels, we built 10 replicate models using the cross-validation approach, by which we randomly split occurrence points into 10 equal-size groups, and ran models 10 times leaving one group out in turn for testing (Elith *et al.*, 2011). For species known to occupy 3–9 pixels, we used a similar cross-validation approach but with two replicates per species instead of 10. To quantify model performance, we obtained the area under the receiver operating characteristic curve (AUC; Fielding & Bell, 1997), which reflects a model's ability to distinguish correctly presence from pseudoabsence (random background points in the study region) for each of the replicated testing datasets (Phillips *et al.*, 2006). AUC ranges from 0 to 1, with AUC = 0.5 suggesting that a model's ability to discriminate presence from pseudoabsence is no better than random. Although MAXENT may have poor performance for species occupying fewer than 5–10 pixels (Wisz *et al.*, 2008), we used it to estimate suitable habitat consistently across all species. MAXENT models built from fewer than 10 occurrences performed well for most species based on AUC (see Table S1.2 in Appendix 1.3). To assess the effects of sample size on estimates of suitable habitat, we randomly subsampled three occupied grid cells from each species 100 times before running MAXENT, and subsequently estimated niche breadth and niche position from this rarefied dataset. When we did so, estimates of niche breadth and position were very similar to those based on all occurrences (niche breadth Pearson's $r = 0.822$, $P < 0.01$; niche position Spearman's rho = 0.817, $P < 0.01$; see Fig. S1.3 in Appendix 1.1), suggesting that MAXENT models of suitable habitat with sample sizes as small as 3 pixels perform reasonably well. Furthermore, results based on analyses

excluding species occupying fewer than 10 grid cells were qualitatively similar to those based on all species (see Figs. S1.4 & S1.5 in Appendix 1.1).

Climatic niche properties

For each species, we quantified niche breadth as the sum of the variances of standardized climatic variables across climatically suitable grid cells, and niche position as the squared difference between the centroid of the multivariate climatic space encompassed by the entire study region and that of climatically suitable pixels (Fig. 2.3c; Martin *et al.*, 2008), using the ADEHABITAT 1.8.12 package (Calenge, 2006) in R 3.0.2 (R Core Team, 2013).

Connectivity of climatically suitable habitat

We measured connectivity among climatically suitable pixels by first creating a minimum spanning tree connecting all pixels of climatically suitable habitat for each species in the NNCLUST 2.2 package (Fig. 2.3d; Lumley, 2010) in R. We then estimated the mean length (in kilometres) of the edges (segments) of the minimum spanning tree, such that shorter distances represent higher connectivity among climatically suitable pixels. We multiplied distances by –1 so that more negative values represented lower connectivity while less negative values represented higher connectivity. We chose this measure of habitat connectivity because it is computationally feasible and need not be influenced by the amount of climatically suitable habitat.

Amount and occupancy of climatically suitable habitat

We estimated the amount of climatically suitable habitat in the study region as the number of climatically suitable pixels for each species (Fig. 2.3b) using the RASTER 2.1–66 package (Hijmans, 2013) in R. We divided the number of climatically suitable pixels in which each species is known to occur (based on point occurrence data) by the amount of climatically

suitable habitat to obtain an estimate of occupancy of suitable habitat (Fig. 2.3b). This method may underestimate occupancy, but assuming that a species occupies every pixel of climatically suitable habitat within its extent of occurrence (described below) would overestimate occupancy; the true occupancy probably lies somewhere between these two extremes.

Geographical range size

We used three metrics of range size that quantify the overall geographical spread of each species. First, we estimated global geographical range size as the extent of occurrence, which measures the spatial extent of the areas occupied by a species (Gaston, 1994). We estimated the extent of occurrence by computing the area of a minimum convex polygon in km^2 encompassing the known occurrences of each species (Fig. 2.3e). Our second and third metrics were the latitudinal and longitudinal extents encompassed by the occurrence points of each species. The three estimates were highly correlated (see Tables S1.3 & S1.4 in Appendix 1.3) and yielded qualitatively similar results, so for simplicity we only present the results for range size estimated as the extent of occurrence.

Controlling for phylogenetic non-independence

Because *Mimulus* species share a recent evolutionary history and thus may not be statistically independent, we tested the assumption of phylogenetic independence for all explanatory and response variables to determine whether phylogenetically based comparative analyses were needed (Abouheif, 1999). We used the phylogeny published in Grossenbacher & Whittall (2011), which is a Bayesian analysis of nuclear ribosomal ITS and ETS and chloroplast *trnL–F* regions from Beardsley *et al.* (2004), concatenated with chloroplast *rpl16* sequences for the *M. moschatus* alliance (Whittall *et al.*, 2006). Of the 72 *Mimulus* species in our analyses, 68 were sampled in the Grossenbacher & Whittall (2011) phylogeny. We then tested the

phylogenetic signal using four widely used methods (see Appendix 1.2). Because tests of phylogenetic signal may fail to detect phylogenetic non-independence, we also performed simple linear regressions on phylogenetically independent contrasts (Felsenstein, 1985) to test for significant relationships between each pair of explanatory and response variables shown in Fig. 2.2b (see Appendix 1.2). We then compared the results based on contrasts with those based on raw species data.

Path analysis

To evaluate the relative importance of the niche breadth, niche position and colonization ability hypotheses in explaining the variation in range size among species, we created a structural equation model describing a simplified version of Fig. 2.2a in which we excluded intrinsic dispersal ability and colonization ability and examined the effects of connectivity of climatically suitable habitat on occupancy of climatically suitable habitat (Fig. 2.2b). Explanatory and response variables were transformed to meet normality assumptions and improve model fit (Fig. 2.2b). We used the LAVAAN 0.5–15 package (Rosseel, 2012) in R to obtain path coefficients and assessed the significance at $P < 0.05$ for each path in the simplified version of Fig. 2.2a. Because our data did not meet the assumption of multivariate normality (multivariate Shapiro–Wilk's test, $P < 0.001$, obtained in MVNORMTEST 0.1–9 package in R; Jarek, 2012), we used the maximum likelihood to estimate model parameters with robust standard errors, and we used a Satorra–Bentler scaled chi-square test statistic to determine whether the covariance matrix observed in our data significantly deviated from that predicted by the structural equation model (Grace, 2006). We ran analyses in a number of different ways, including with and without outliers or taxa undergoing major revision, bootstrapping versus Satorra–Bentler scaled χ^2 and several transformations of variables, and in all cases the observed covariance matrix in our data differed

significantly from the model predictions (Satorra–Bentler corrected $\chi^2 = 33.811$, d.f. = 7, $P < 0.01$). Thus we took an exploratory approach by inspecting modification indices to assess which biologically plausible paths needed to be added to achieve an adequate model fit (see Appendix 1.2; Grace, 2006). With this process, we arrived at a modified model including correlations between the amount of suitable habitat and connectivity, and between niche breadth and occupancy of suitable habitat (Fig. 2.2b, and see Appendix 1.2). The observed covariance matrix from our dataset did not deviate significantly from the modified model (Satorra–Bentler corrected $\chi^2 = 6.883$, d.f. = 5, $P = 0.229$; Fig. 2.2b). We used estimates of standardized path coefficients and R^2 for each endogenous variable from this resulting modified model to assess the relative importance of each hypothesis.

Null model

Because the geographical ranges of widespread species may encompass greater climatic variation than geographically restricted species simply by chance, observed relationships between range size, niche properties, connectivity and the amount and occupancy of suitable habitat may be artefacts (Gaston, 2003; Davies *et al.*, 2009). To address this issue, we used a null model that randomized the location of species' geographical ranges across the study region, while preserving the spatial structure of the occurrence data (see details in Appendix 1.2). We used this null model to create 100 datasets, each including all the variables in the modified structural equation model (Fig. 2.2b). We then fit this modified structural equation model to each null dataset. We estimated the 95% confidence interval for the Satorra–Bentler corrected χ^2 and each of the path coefficients derived from the null datasets by calculating percentiles of the distribution of path coefficients. We used one-tailed 95% confidence intervals for the Satorra–Bentler corrected χ^2 because the observed data should have a better model fit than the null

datasets, and we used two-tailed 95% confidence intervals for all path coefficients. If a path coefficient from the observed data fell outside the respective confidence interval of the distribution of path coefficients derived from null datasets, we concluded that the observed path coefficient was significantly different from the null model expectation.

Results

Performance of climatic niche models

Cross-validation AUC for test data indicated that MAXENT models performed better than random models for all species, with most species having a mean test AUC > 0.9 across cross-validation replicates (Table S2.2 in Appendix 1.3). *Mimulus calciphilus* had a mean test AUC < 0.75, probably because it was only documented in 3 pixels and only one or two occurrences were used to train or test replicate models.

Phylogenetic non-independence

We did not detect a significant phylogenetic signal in niche breadth, niche position, connectivity, amount of suitable habitat or extent of occurrence ($P > 0.05$), with a white noise non-phylogenetic model of evolution having the lowest sample size-corrected Akaike information criterion (AIC_c; Table 2.1). We detected a weak phylogenetic signal in occupancy of suitable habitat when testing for a phylogenetic signal based on the Abouheif's test (Abouheif 1999), with an Ornstein–Uhlenbeck model (Hansen 1997) having a slightly lower AIC_c than the white noise model of evolution (Table 2.1). All correlations and simple regressions performed on raw species data were qualitatively similar to those performed on phylogenetically independent contrasts (see Tables S1.3–S1.6 in Appendix 1.3 and Figs. S1.5 & S1.6 in Appendix 1.1).

Path analyses and null model

The observed structural equation model test statistic fell outside the 95% confidence interval of the distribution of test statistics derived from null datasets (Fig. 2.4a), representing a better model fit than the null model expectation. Together, niche breadth and niche position explained nearly 89% of the variation in amount of climatically suitable habitat, with niche breadth explaining more variation than niche position (niche breadth → amount of suitable habitat standardized path coefficient = 0.840, versus niche position → amount of suitable habitat standardized path coefficient = –0.328; Fig. 2.2b). As predicted, niche breadth had a positive effect and niche position had a negative effect on the amount of suitable habitat (Fig. 2.2b). While the observed path coefficient from niche breadth to amount of suitable habitat was greater than expected from the null model (Fig. 2.4b), the observed path coefficient from niche position to amount of suitable habitat was not (Fig. 2.4c). Contrary to prediction, connectivity had a negative effect on occupancy of suitable habitat, explaining 13% of the variation in occupancy of suitable habitat (Fig. 2.2b), but the observed path coefficient was not distinguishable from the null model expectation (Fig. 2.4d).

As predicted, the amount and occupancy of suitable habitat had a positive effect on range size, together explaining 83% of the variation in range size, with the amount of suitable habitat explaining more variation than occupancy of suitable habitat (amount of suitable habitat → geographical range size standardized path coefficient = 1.098, versus occupancy of suitable habitat → geographical range size standardized path coefficient = 0.683; Fig. 2.2b). Furthermore, the path coefficients from both amount and occupancy of suitable habitat to geographical range size were greater than expected from the null model (Fig. 2.4e, f). Consistent with null model expectations, niche breadth and niche position were not correlated (Fig. 2.2b &

Fig. 2.4g). In addition to the predicted relationships, we also detected a positive relationship between niche breadth and connectivity, a negative relationship between niche position and connectivity, and a positive relationship between amount of suitable habitat and connectivity (Fig. 2.2b), but none of these path coefficients deviated from null model expectations (Fig. 2.4h–j). We also documented an unexpected negative relationship between niche breadth and occupancy of suitable habitat; this path coefficient was more negative than expected from the null model (Fig. 2.4k).

Discussion

Despite conspicuous variation in geographical range size among species, few studies have shed light on the relative importance of the multiple mechanisms that may drive such variation. We used western North American monkeyflowers to assess the relative importance of climatic niche properties and connectivity of climatically suitable habitat in determining variation in range size among species. Niche breadth and position explained more than half of the variation in amount of climatically suitable habitat, with niche breadth having a greater effect than niche position. Moreover, the effect of niche breadth on amount of suitable habitat was greater than expected from the null model that randomized geographical ranges across the study region, while the effect of niche position on amount of climatically suitable habitat failed to differ from the null model expectation. The amount of climatically suitable habitat, in turn, explained much of the variation in range size, whereas the occupancy of climatically suitable habitat explained a smaller portion of the variation in range size. These effects of amount and occupancy of climatically suitable habitat on range size were larger than null model expectations. Our metric of connectivity did not have a positive effect on occupancy of suitable habitat, thereby failing to support one prediction of the colonization ability hypothesis. Finally, the

covariance structure of the data yielded an unforeseen relationship between niche breadth and occupancy of suitable habitat, suggesting that niche breadth and occupancy interact to shape the geographical range sizes of western North American monkeyflower species. Below we assess the evidence relevant to each of the mechanistic hypotheses we examined, and draw conclusions about the relative roles of climatic niche breadth and position, connectivity of climatically suitable habitat, and amount and occupancy of climatically suitable habitat, in explaining variation in range size among species.

Climatic niche properties

We found that climatic niche breadth was the strongest predictor of range size in western North American monkeyflowers. Although many studies have detected a positive relationship between niche breadth (or climatic tolerance) and range size (e.g. Pither, 2003), few have corrected for possible effects of range size on estimates of niche breadth, and the extent to which this potential bias has confounded the results of many studies remains unclear. Our study adds to the growing set of results showing that a positive relationship between niche breadth and range size is not artefactual (Fig. 2.4b; reviewed in Slatyer *et al.*, 2013). Our results contrast with studies finding that niche position is a better predictor of occupancy than niche breadth (Heino & Soininen, 2006; Hurlbert & White, 2007). We found that the relationship between niche position and range size can be explained by a null model that randomly places geographical ranges across the study region (Fig. 2.4c), in contrast with other findings documenting support for the niche position hypothesis (Gregory & Gaston, 2000; Heino, 2005; Heino & Soininen, 2006; Hurlbert & White, 2007). However, the way we delineated the study region prevented geographically widespread species from having a high niche position. This geometric constraint of species with large ranges might have been alleviated if we had used a larger study region. It may be easier to

detect an effect of niche position that is not explained by a null model that preserves range size for clades containing mostly allopatric species, because the reference study region would be quite large. Unlike previous tests of niche breadth and position hypotheses, we have shown that the predicted effects of niche breadth and position on range size are mediated by the amount of suitable habitat (Fig. 2.2b), thereby improving our understanding of the mechanisms underlying patterns of variation in range size. Niche breadth and position were not strongly correlated in our final path analysis (Fig. 2.2b), suggesting that they constitute two rather independent axes of rarity. Nonetheless, our results suggest that Rabinowitz's (1981) form of rarity, in which a species has both a small geographical range and a narrow niche, may be more common than other forms.

Connectivity of climatically suitable habitat

Although occupancy of suitable habitat explained some variation in range size among species, our metric of connectivity was not a good predictor of occupancy of suitable habitat. Bivariate results suggested that the negative effect of connectivity on occupancy of suitable habitat might have been driven by outlier species with high occupancy and low connectivity (see Fig. S1.5f in Appendix 1.1). Although we focused on the connectivity of climatically suitable habitat as measured by the average edge length of minimum spanning trees, there are numerous ways to estimate such connectivity (Fortin & Dale, 2005). Furthermore, while we focused on extrinsic climatic factors that affect colonization ability, other factors such as the diversity of life-history strategies, mating systems, edaphic specializations and habits encompassed by *Mimulus* species (Wu *et al.*, 2008) may outweigh the effects of the connectivity of climatically suitable habitat on the overall colonization ability of species. In the future, it would be beneficial to obtain information on traits associated with species' intrinsic colonization abilities, such as

selfing rates (Randle *et al.*, 2009) and seed size (Morin & Chuine, 2006), particularly in light of several examples of dispersal ability being a better predictor of geographical range size than niche properties (Bohning-Gaese *et al.*, 2006; Kristiansen *et al.*, 2009; Blanchet *et al.*, 2013; Laube *et al.*, 2013). There are, however, many circumstances under which dispersal ability need not correlate with geographical range size. For instance, climatically suitable habitat may not be highly fragmented for most *Mimulus* species, as indicated by the small range of connectivity values (see Fig. S1.5f, h in Appendix 1.1). Given that occupancy of climatically suitable habitat had a positive effect on range size despite the lack of support for a positive relationship between connectivity and occupancy of climatically suitable habitat, occasional long-distance dispersal events may be more important than connectivity in determining the occupancy of suitable habitat (Lester *et al.*, 2007). However, we emphasize the need to interpret the occupancy results with caution because, without absence data, estimates of occupancy may suffer from collection biases that could potentially result in reshuffling of the relative ranks of estimated occupancy relative to true occupancy (Sheth *et al.*, 2012).

Other determinants of geographical range size

Although we found strong support for the niche breadth hypothesis, our study did not include other potential determinants of geographical range size and occupancy. In our study, we focused on the climatic niche, but other niche axes, such as edaphic properties, could also influence species distributions. Some studies have documented a positive relationship between species age and range size (e.g. Jablonski, 1987; Webb & Gaston, 2000; Paul *et al.*, 2009), suggesting that over time species are able to fill more of their available niche space and/or adapt and expand into novel niche space, thus achieving broader realized niches and larger range sizes. If species age were driving variation in range size among *Mimulus* species, then the positive

relationship we detected between niche breadth and range size could be a result of younger species having narrower niches and thus smaller ranges than older species. Furthermore, the speciation rate within a particular clade could influence the range sizes of species in that clade, such that clades with higher speciation rates may tend to have more species with smaller ranges than clades with low speciation rates (Lester & Ruttenberg, 2005). However, if variation in speciation rates among clades within *Mimulus* were driving the variation in range size, then we should have detected a phylogenetic signal in range size as a result of certain clades with high speciation rates having species with small geographical ranges. Recent work suggests that the patterns of niche breadth and range size in western North American *Mimulus* support a budding mode of speciation (Grossenbacher *et al.*, 2014), which may explain the lack of phylogenetic signal in range size.

Conclusions

In this study we have shown that climatic niche breadth explained more variation in geographical range size among *Mimulus* species than niche position and connectivity of climatically suitable habitat. The results of our study contribute to disentangling the mechanisms underlying patterns of variation in range size among species by providing empirical support for the idea that climatic niche breadth, via its effect on the amount of suitable habitat, drives variation in range size in western North American monkeyflowers, despite other differences among species (e.g. edaphic substrate and mating system) and other causes of range-size variation (e.g. evolutionary and biogeographical history). To understand further the mechanisms underlying the niche breadth hypothesis, experiments assessing whether wide-ranging species have broader niches than narrowly distributed species would be useful. For example, experiments in environmental chambers would allow tests of whether *Mimulus* species with large

geographical ranges have broader thermal performance breadths than narrowly distributed relatives. In sum, we have shown that climatic niche breadth influences species' rarity, and thus may constitute a major axis of extinction risk. Consequently, by improving our understanding of the processes driving patterns of rarity, this study increases our ability to assess species' vulnerabilities to extinction.

Table 2.1 Tests for significant phylogenetic signal in the variables included in the structural equation model (Fig. 2.2b) for western North American *Mimulus* species. AIC_c, sample size-corrected Akaike information criterion for Brownian motion (BM), Ornstein–Uhlenbeck (OU) and white noise (WN) models of evolution, with the lowest AIC shown in bold. See Appendix 1.2 for detailed methods.

Variable	Abouheif's C_{mean}	Pagel's λ	Blomberg's K	BM AIC_c	OU AIC_c	WN AIC_c
Niche $breadth^{0.25}$	–0.039	0.077	0.039	90.87	–9.60	**–11.78**
Niche position	0.037	6.641×10^{-5}	0.085	373.52	326.68	**325.85**
Connectivity	–0.079	6.641×10^{-5}	0.036	378.92	275.36	**273.26**
Log_{10} (amount of suitable habitat)	–0.072	0.033	0.039	247.15	146.73	**144.54**
Log_{10} (occupancy of suitable habitat)	0.171*	6.641×10^{-5}	0.046	172.07	**120.15**	120.57
Log_{10} (geographical range size)	–0.166	6.641×10^{-5}	0.034	317.42	209.58	**207.39**

*Significant phylogenetic signal at $P < 0.05$.

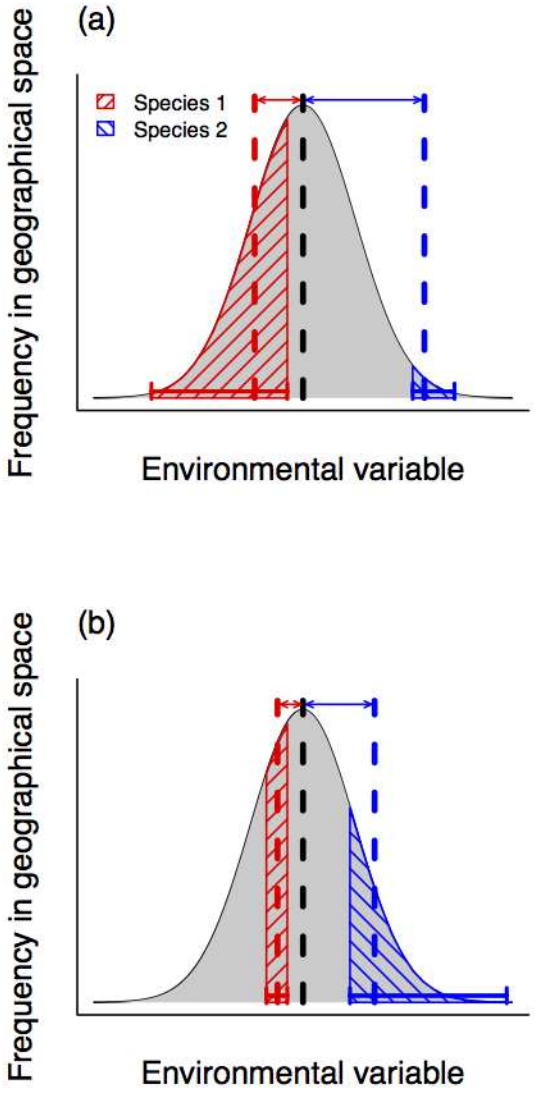

Figure 2.1 (a) The frequency of environments (grey area) and amount of suitable habitat for two hypothetical species (hatched areas) across a hypothetical study region. Dashed lines indicate the mean environmental values across the study region and across the niche of each species. Arrows indicate the niche position for each species. Species 1 encompasses more variation along the environmental axis (the solid horizontal line immediately above the *x*-axis) and has more suitable habitat (hatched area) than species 2, a pattern that is consistent with the niche breadth hypothesis. Species 1 also has a mean environmental value that is closer to the average environmental conditions across the study region than the mean environmental value of species 2, so the difference in amount of suitable habitat between species 1 and 2 is also consistent with the niche position hypothesis. The niche breadth and niche position hypotheses predict that increasing amount of suitable habitat increases geographical range size (Fig. 2.2a). (b) Niche breadth and position need not be negatively correlated, as a species could have a narrow niche and a low niche position (species 1), or a broad niche and a high niche position (species 2).

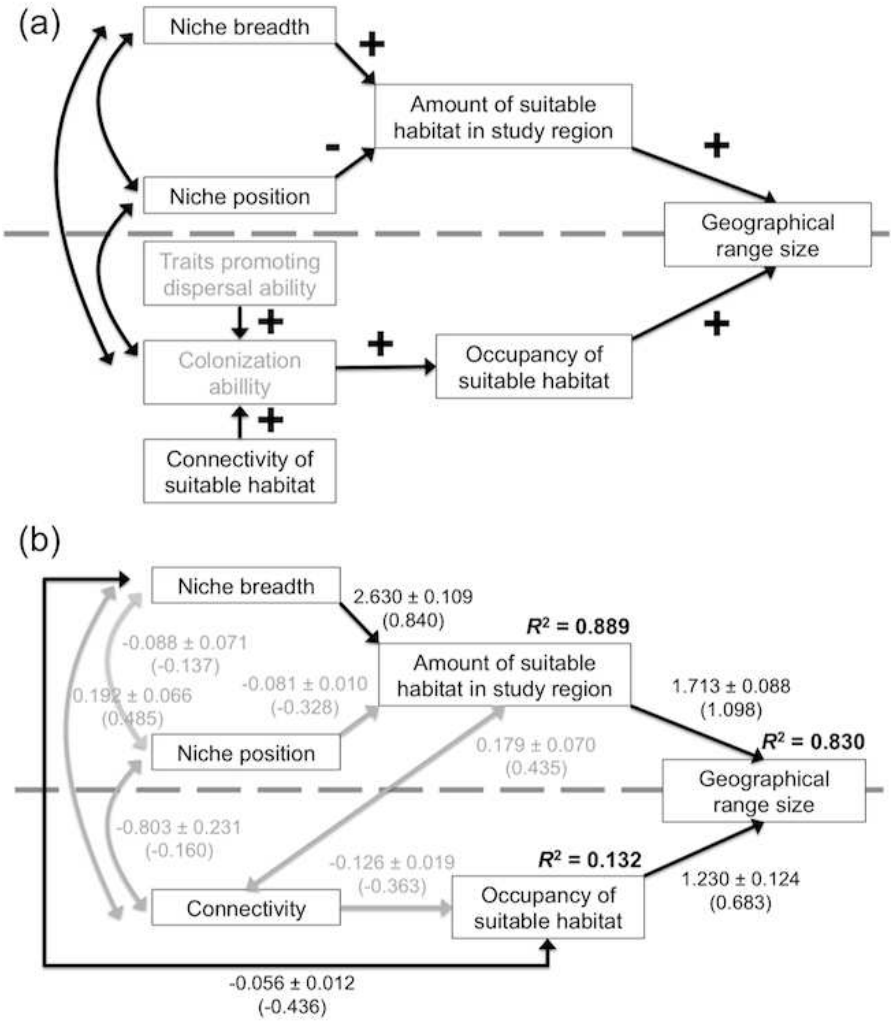

Figure 2.2 (a) Conceptual diagram of how niche properties (above the dashed line) and colonization ability (below the dashed line) are hypothesized to influence geographical range size. Single-headed arrows indicate causal relationships, double-headed arrows indicate correlations and grey labels correspond to variables that were not measured directly in our study. (b) A modified structural equation model used to assess the relative importance of climatic niche properties (above the dashed line) and connectivity of climatically suitable habitat (below the dashed line) in explaining variation in geographical range size among western North American *Mimulus* species. Unstandardized regression coefficients ± 1 standard error are shown for each arrow, with standardized coefficients in parentheses. Black arrows represent significant path coefficients and grey arrows represent non-significant path coefficients at an $\alpha = 0.05$ significance level according to the test based on the null model (see Fig. 2.4). R^2-values above each endogenous variable indicate the amount of variation explained by the model. We transformed niche breadth to the fourth root, and we log-transformed the amount and occupancy of suitable habitat and geographical range size.

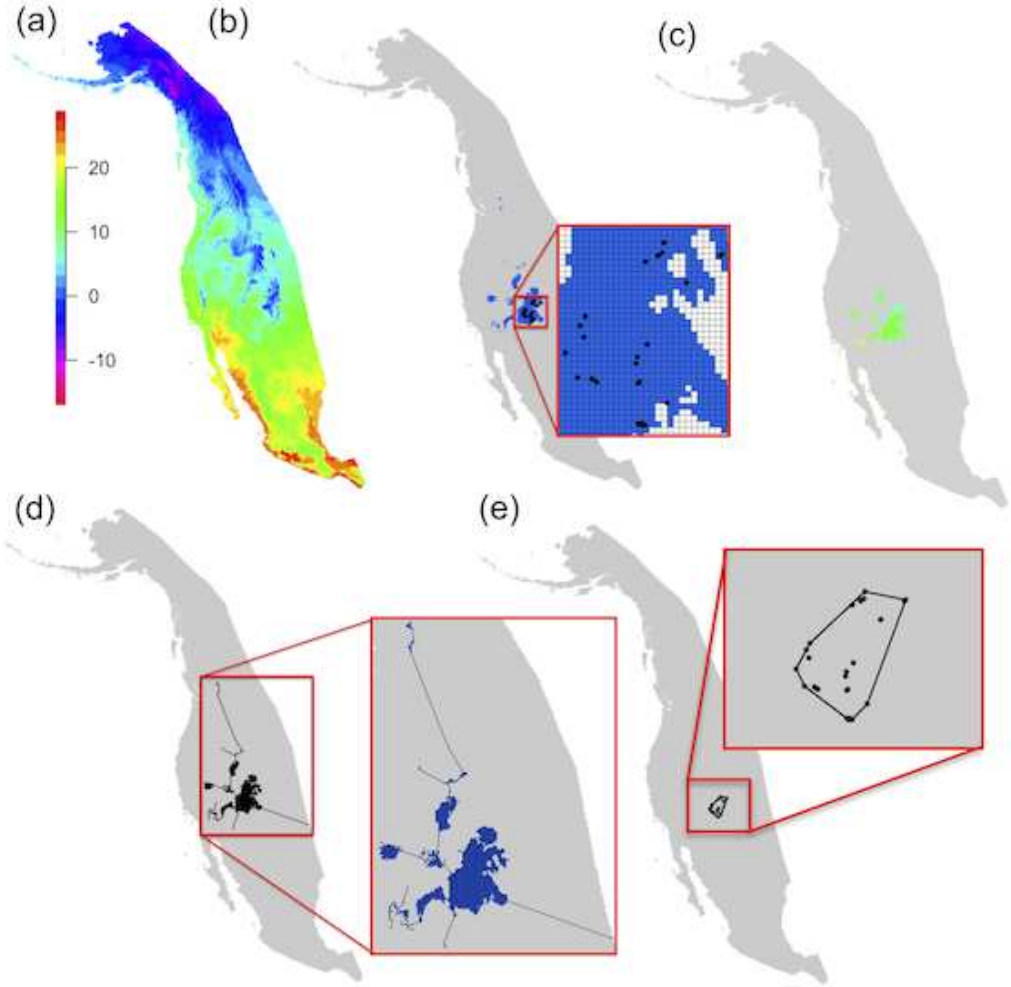

Figure 2.3 Illustration of how locality records and climatic data were used to estimate range size, climatic niche properties and connectivity of climatically suitable habitat for one species, *Mimulus eastwoodiae*, in western North America. (a) Mean annual temperature (°C), one of seven climatic variables used to estimate niche breadth, across the study region in western North America. (b) The proportion of the total number of climatically suitable pixels (in blue; see Materials and Methods for the definition of climatically suitable habitat) that are occupied based on herbarium specimen data (black points). (c) Mean annual temperature across climatically suitable pixels. Niche position is the difference between the mean temperature across the study region (a) and the mean temperature across climatically suitable pixels of a given species (c) but in multivariate climatic space. (d) Minimum spanning tree connecting climatically suitable pixels (used to estimate connectivity). (e) Extent of occurrence based on a minimum convex polygon (black outline) connecting all herbarium specimen data (black points).

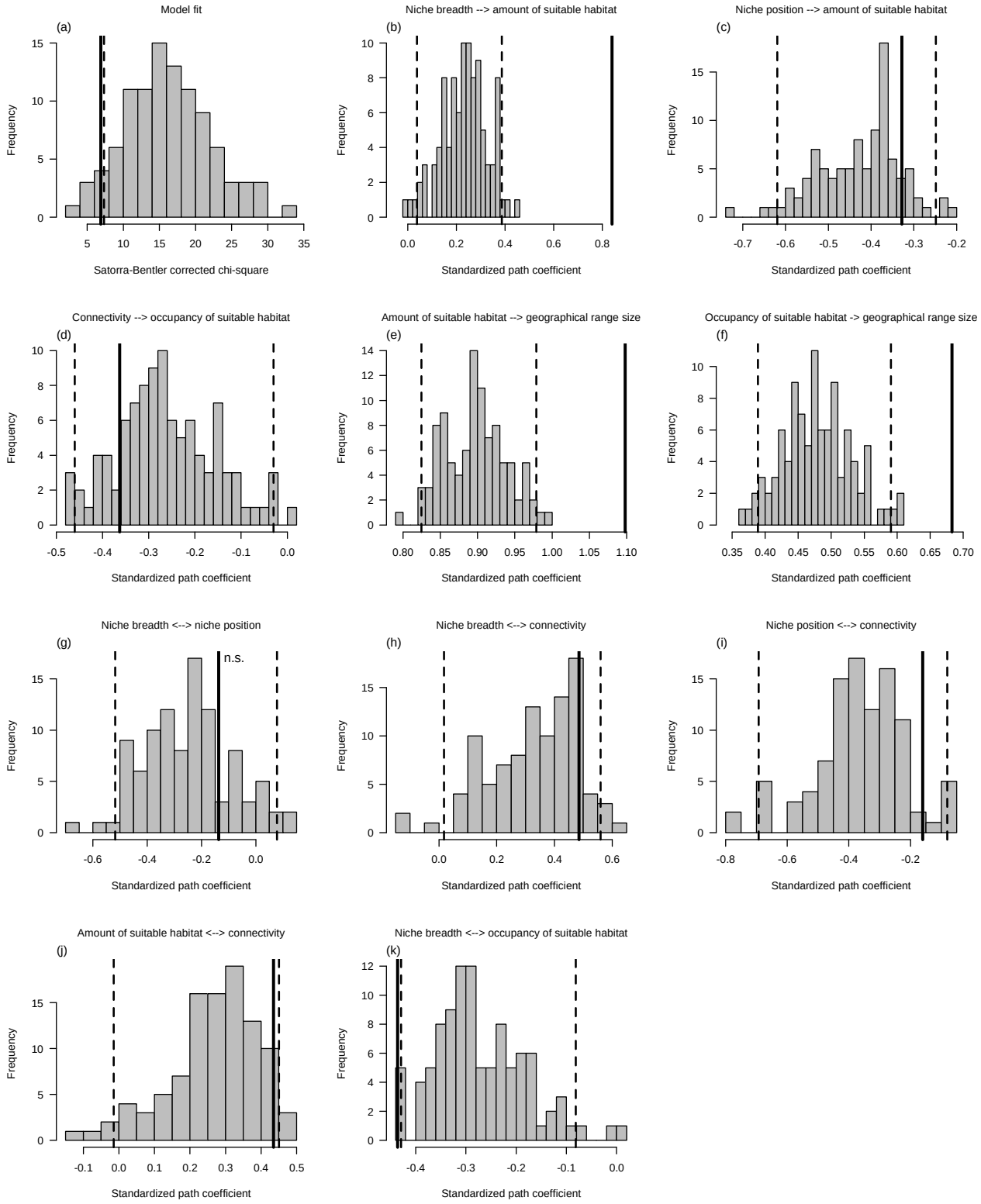

Figure 2.4 Frequency distributions of Satorra–Bentler corrected χ^2 (a) and path coefficients (b–k) derived from 100 null datasets (see Materials and Methods and Appendix 1.2 for details), with

dashed lines representing the respective 95% confidence intervals and the solid lines representing the test statistic or path coefficients derived from observed data for western North American *Mimulus* species. The observed path coefficient denoted as n.s. in (g) was not significantly different from 0 ($P = 0.212$).

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3. THE EVOLUTION OF ENVIRONMENTAL TOLERANCE AND RANGE SIZE: A COMPARISON OF GEOGRAPHICALLY RESTRICTED AND WIDESPREAD *MIMULUS*[1]

Summary

The geographic ranges of closely related species can vary dramatically, yet we do not fully grasp the mechanisms underlying such variation. The niche breadth hypothesis posits that species that have evolved broad environmental tolerances can achieve larger geographic ranges than species with narrow environmental tolerances. In turn, plasticity and genetic variation in ecologically important traits and adaptation to environmentally variable areas can facilitate the evolution of broad environmental tolerance. We used five pairs of western North American monkeyflowers to experimentally test these ideas by quantifying performance across eight temperature regimes. In four species pairs, species with broader thermal tolerances had larger geographic ranges, supporting the niche breadth hypothesis. As predicted, species with broader thermal tolerances also had more within-population genetic variation in thermal reaction norms and experienced greater thermal variation across their geographic ranges than species with narrow thermal tolerances. Species with narrow thermal tolerance may be particularly vulnerable to changing climatic conditions due to lack of plasticity and insufficient genetic variation to respond to novel selection pressures. Conversely, species experiencing high variation in temperature across their ranges may be buffered against extinction due to climatic changes because they have evolved tolerance to a broad range of temperatures.

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Introduction

Geographic range size can vary by orders of magnitude among species in the same clade (Darwin 1859), among clades, and predictably across geography (e.g. Rapoport's rule; Stevens 1989), yet we do not fully grasp the mechanisms underlying such variation. Numerous hypotheses have been invoked to explain variation in range size (reviewed in Gaston 2003), but in particular explanations for variation in range size among closely related species and across space have focused on the evolution of niche breadth (Pither 2003; Slatyer et al. 2013). Some have regarded a species' geographic range as a projection of the ecological niche onto geography (Pulliam 2000). The ecological niche can be viewed as the set of environments across which a species can maintain viable populations (Hutchinson 1957). The niche breadth hypothesis posits that, all else being equal, species that are able to maintain viable populations across a greater set of environments can achieve larger geographic ranges than species with narrow ecological niches (Fig. 3.1a, b; Brown 1984). This hypothesis has garnered consistent support, suggesting that a positive relationship between niche breadth and geographic range size is a general pattern (Slatyer et al. 2013). This relationship is particularly strong when quantifying niche breadth as environmental tolerance, defined as the range of abiotic conditions (e.g., temperature) across which performance is high (Slatyer et al. 2013).

A species can accrue environmental tolerance in a number of ways. First, a species with broad environmental tolerance may be composed of phenotypically plastic genotypes (Baker 1965) that perform well across a broad range of environmental conditions (Fig. 3.1c). For example, phenotypic plasticity, rather than local adaptation, has allowed the weed *Verbascum thapsus* to invade high elevations in California (Parker et al. 2003). Second, adaptively differentiated individuals within a population may shape a species' environmental tolerance,

such that populations of species with broad environmental tolerances consist of many divergently specialized individuals differing in environmental optima (Bolnick et al. 2003). In fact, there is evidence that individual specialization can explain a large fraction of a population's total niche breadth across a variety of taxa (Bolnick et al. 2003). Third, a species may achieve broad environmental tolerance via local adaptation of divergent populations to a range of environments (Ackerly 2003). For example, each population of lodgepole pine (*Pinus contorta*) is locally adapted to a subset of climates occupied by the species as a whole, such that the broad climatic tolerance exhibited by the species can be partitioned among populations (Rehfeldt et al. 1999). Thus, variation in environmental performance within and among genotypes, families, or populations may play an important role in shaping species-level niche breadth (Slatyer et al. 2013), and these alternative means for achieving broad environmental tolerance have important implications for understanding variation in evolutionary potential of populations and species (Etterson 2008).

Variation in environmental tolerance among species may arise due to constraints on the evolution of broad environmental tolerances. One constraint may arise due to a lack of genetic variation in traits that would permit range expansion via adaptation to novel environments (Kellermann et al. 2009). If so, species with narrower environmental tolerances may have less genetic variation for environmental tolerance and thus have smaller geographic ranges than species with broader environmental tolerances (Fig. 3.1d). Another explanation for constraints to evolving a broader environmental tolerance deals with fitness tradeoffs (Futuyma and Moreno 1988). Theory predicts tradeoffs between environmental tolerance and maximum fitness, such that there is a cost in maximum fitness to having a broad environmental tolerance (Huey and Hertz 1984). If a specialist-generalist tradeoff is present among species and "a jack-of-all-trades

is a master-of-none" (MacArthur 1972), then on average, species with broad environmental tolerances should have a lower maximum fitness or performance metric than species with narrow niches (Huey and Slatkin 1976; Fig. 3.1b, e).

In addition to intrinsic constraints within species, extrinsic factors such as geographically variable selection pressures may also shape environmental tolerance. In particular, the climatic variability hypothesis invokes variation in natural selection across space to posit that species that have adapted to climatically variable environments such as temperate zones have evolved broader climatic tolerances and should thus be able to occupy larger geographic ranges than species occurring in climatically stable environments such as the tropics (Janzen 1967; Stevens 1989). Although in its original form, this hypothesis focused on temporal climatic variability within a site, this hypothesis also predicts that species with ranges encompassing greater variation in climate should have broader environmental tolerances and larger geographic ranges than species experiencing less variation in climate across their ranges (Quintero and Wiens 2013; Fig. 3.1e). The climatic variability hypothesis has been invoked to explain Rapoport's rule, the pattern of average range size in a clade decreasing from temperate to tropical areas (Stevens 1989), as well as latitudinal gradients in biodiversity (Ghalambor et al. 2006), but it can be applied more generally to species that differ in the climatic variability experienced across their ranges. Depending on the relationship between selection and gene flow (Lenormand 2002), climatic variability across species' ranges could favor locally adapted populations, phenotypic plasticity (Fig. 3.1c), within-population genetic variation in climatic tolerance (Fig. 3.1d), or a combination of strategies leading to an overall broad species-level environmental tolerance.

In this study, we examine the ideas outlined above (Fig. 3.1e) in western North American monkeyflowers (genus *Mimulus*, renamed *Erythranthe* in Barker et al. 2012). We focus on one

niche axis, temperature, which affects a number of physiological processes in living organisms (Angilletta 2009). Specifically, we experimentally quantified thermal performance breadth for five pairs of closely related species that differ in geographic range size. First, we evaluated the hypothesis that geographically widespread species have wider thermal performance breadths than geographically restricted species (Fig. 3.1a). Second, we determined whether species achieve broad thermal tolerance via phenotypically plastic genotypes (Fig. 3.1c). Third, we tested whether genetic variation in thermal reaction norms increases thermal tolerance (Fig. 3.1d), and examined whether specialist-generalist tradeoffs (Fig. 3.1b) shape patterns of thermal tolerance. Finally, we assessed the prediction of the climatic variability hypothesis that species with broader thermal tolerance experience greater variation in temperature across their geographic ranges than species with narrow thermal tolerance (Fig. 3.1e).

Methods

Study system

The objectives of this research were addressed with the monkeyflower genus *Mimulus* (Phrymaceae), a group of wildflowers with ~ 90 species in western North America (Beardsley and Olmstead 2002). Western North American *Mimulus* is in the process of taxonomic revision (Barker et al. 2012), but the anticipated modifications are predominantly nomenclatural and should not affect the species identity of the populations in our study. *Mimulus* species occur in several habitats, including wetlands, alpine environments, and deserts, and some species are edaphic specialists (Wu et al. 2008). Further, *Mimulus* species encompass herbaceous and woody habits, annuals and perennials, and mating systems ranging from complete outcrossing to obligate selfing (Wu et al. 2008). Due to its short generation times (6-12 weeks), ease of propagation, high seed production, and genomic resources, *Mimulus* has become an emerging

model system in evolutionary ecology (Wu et al. 2008). The geographic ranges of *Mimulus* species are well-known, occur primarily within protected areas in western North America, and exhibit strong variation in range size (Beardsley et al. 2004), thus constituting an appropriate study system for testing hypotheses about relationships between range size and thermal tolerance. Previous work suggests that *Mimulus* species exhibit substantial variation in climatic niche breadth (Sheth et al., in press), with some species possessing significant genetic variation for climatic tolerance (Vickery 1972).

Field sampling and crosses

To test hypotheses about relationships among geographic range size, environmental tolerance, and processes shaping environmental tolerance (Fig. 3.1e), we focused on five species pairs that broadly sample the western North American *Mimulus* phylogeny and consist either of putative sister species or of species within a single subclade of *Mimulus* (Beardsley et al. 2004; Table 3.1). We selected pairs in which species differ markedly in range size (Sheth et al., in revision) and are amenable to greenhouse study (Hiesey et al. 1971; Sobel 2010). Comparison of close relatives allows for comparisons of traits among widely and narrowly distributed species pairs and prevents drivers of variation of range size from being masked by differences that have accumulated over long periods of independent evolution. For each species, we collected seeds from 20 to 50 individuals at a single site, collecting where species in a given pair either co-occur at a site (Fig. 3.2a, e) or are at least regionally sympatric (Fig. 3.2b-d). This sampling scheme of one population per species yields a conservative test of the niche breadth hypothesis by assuming that there are innate species-level differences in niche breadth, and avoids potential confounding of local adaptation and spatial distance among multiple populations of widespread vs. rare species. We planted field-collected seeds from each species in the Colorado State University

Greenhouse. For the species with focal populations that are predominantly outcrossing (*M. cardinalis, M. verbenaceus, M. eastwoodiae, M. bicolor, M. filicaulis,* and *M. guttatus*; Sheth, unpubl. data), we randomly crossed individuals within each species to produce outcrossed seeds with which to conduct controlled experiments. For the species with focal populations that are predominantly selfing (*M. parishii, M. floribundus, M. norrisii,* and *M. laciniatus*; Sheth, unpubl. data), we allowed individuals to self for one generation and used the resulting seeds in subsequent experiments. We used this crossing scheme because it best mimics what is occurring in natural populations. If we had created outcrossed seeds from predominantly selfing species, the resulting estimates of thermal performance breadth and genetic variation in thermal reaction norms could have been inflated, failing to correspond to what actually occurs in the wild. A single *Mimulus* fruit typically contains hundreds of seeds, so we used the outcrossed or selfed seeds from the same full-sibling seed families in all experiments described below (see Table 3.2 for number of families per species).

Plant propagation

We established seedlings of all study species in 72-cell plug trays (4 x 4 x 5.5 cm). For *M. cardinalis, M. parishii, M. verbenaceus, M. eastwoodiae, M. guttatus,* and *M. laciniatus,* we filled plug trays with Farfard 4P Mix potting soil with a thin layer of Farfard Superfine Germinating mix on top (Conrad Farfard, Inc., Agawam, MA, USA). *Mimulus guttatus* and *M. laciniatus* were first placed in a refrigerator at 4^{o}C for 10 days to improve germination success prior to being moved to the Colorado State University Greenhouse. For *M. floribundus, M. norrisii, M. bicolor,* and *M. filicaulis,* we treated seed with gibberellic acid (Acros Organics) to improve germination success. In particular, we soaked seeds in .2 mM giberellic acid solution in 1.5 mL microcentrifuge tubes for ~8 hours and then rinsed seeds thoroughly with dH_2O to

minimize potential downstream effects of gibberellic acid on growth (Bachelard 1968). Subsequently, we planted seeds into a mix of 3 parts potting soil and 2 parts perlite with a thin layer of germination mix on top. Prior to being placed in growth chambers for thermal performance experiments, plug trays were kept in the Colorado State University Greenhouse with a 16 h day/8 h night photoperiod with day temperature programmed to ~25^{o}C and night temperature at ~20^{o}C.

Thermal performance experiments

Temperature is one niche dimension that affects fitness components in *Mimulus*. For example, temperature affects whole-plant performance of *M. cardinalis* and *M. lewisii* (Angert 2006), and the species pairs we chose differ in latitudinal distributions and in the range of temperatures experienced within these distributions (Table 3.1). We measured survival and relative growth rate (*RGR*) of individuals of each species across eight temperature regimes simulated in growth chambers with 14 hours of daylight and 10 hours of darkness per 24 hour period according to these day/night temperatures (^{o}C): 15/0, 20/5, 25/10, 30/15, 35/20, 40/25, 45/30, and 50/35 (based on the range of temperatures experienced by western North American *Mimulus*). Relative growth rate constitutes one of many possible measures of performance, and we chose it because it was the most feasible performance metric to estimate for thousands of plants. Although *RGR* need not be correlated with lifetime fitness, there is evidence for many of our study species that as *RGR* increases, flower number increases (Weimer & Sheth, unpubl. data). Further, rapid growth at early life stages during which plants are smaller and more vulnerable should increase the chances of juvenile survival and thus should influence the probability that a plant will reproduce.

Experiments were conducted from September 2012 through January 2013. During any given week, 5-10 seeds from each family (11-50 families per species; Table 3.2) of all or a subset of species pairs were sown as described above. Each tray was comprised of both species in a species pair, with families and species completely randomized. Within each growth chamber run, there were two replicate trays per species pair for *M. cardinalis*, *M. parishii*, *M. verbenaceus*, and *M. eastwoodiae*, such that each tray contained one replicate set of families for each species. For *M. floribundus*, *M. norrisii*, *M. bicolor*, *M. filicaulis*, *M. guttatus*, and *M. laciniatus*, there was one tray per species pair, but each tray contained two replicates of each family for each species within each growth chamber run. Thus, each family of each species was replicated twice within each growth chamber run. Once seeds were sown, trays were sub-irrigated daily and rotated three times weekly to reduce positional effects. Two weeks after sowing *M. floribundus*, *M. norrisii*, *M. bicolor*, and *M. filicaulis* and three weeks after sowing *M. cardinalis*, *M. parishii*, *M. verbenaceus*, *M. eastwoodiae*, *M. guttatus*, and *M. laciniatus* seeds, each cell in each plug tray was thinned down to one central-most seedling. Three weeks after sowing *M. floribundus*, *M. norrisii*, *M. bicolor*, and *M. filicaulis* and four weeks after sowing *M. cardinalis*, *M. parishii*, *M. verbenaceus*, *M. eastwoodiae*, *M. guttatus*, and *M. laciniatus* seeds, we measured stem length and leaf number and placed plants into one of two Percival LT-105 growth chambers (Percival Scientific, Inc., Perry, IA, USA) programmed at one of the 8 temperature regimes described above for seven days. Upon being placed into a particular temperature regime, plants ranged from having 2 to 12 leaves, with stem length ranging from 0.1 to 3.5 cm, depending on species. While plants were in growth chambers, we sub-irrigated trays daily and rotated trays within each chamber three times to reduce positional effects. Seven days later, we removed plants from chambers and measured them again to estimate *RGR* in stem

length and leaf number as the change in size per initial size per day. For *M. cardinalis*, *M. parishii*, *M. verbenaceus*, *M. eastwoodiae*, *M. floribundus*, and *M. norrisii*, *RGR* in leaf number varied more predictably with temperature and was thus considered a more relevant estimate of performance, and similarly, *RGR* in stem length was more appropriate for *M. bicolor*, *M. filicaulis*, *M. guttatus*, and *M. laciniatus*. Before going into chambers, *M. filicaulis* and *M. laciniatus* were the only species that ever had floral buds or flowers. In addition, *M. bicolor*, *M. floribundus*, *M. norrisii*, and *M. guttatus* plants sometimes had floral buds or flowers when coming out of the growth chambers.

We replicated these temperature regimes twice for *M. cardinalis*, *M. parishii*, *M. verbenaceus*, and *M. eastwoodiae*, with each temperature replicated in each growth chamber once, except for the 50/35^{o}C temperature regime, which we replicated twice in the same chamber. Due to logistical constraints, we replicated these temperature regimes once (randomly assigning each temperature regime to one of the two growth chambers) for the remaining species. We randomized the order in which we conducted the 8 temperature regimes. During the course of the experiment, we planted a total of 5,960 individuals but 307 individuals did not germinate, resulting in a total of 5,653 individuals that we measured prior to going into a particular temperature treatment. Of these individuals, we excluded 202 individuals that did not have any leaves at least 1 mm long prior to exposure to a particular temperature treatment, resulting in a total of 5,451 plants used for estimating thermal performance curves. For 364 individuals that did not survive after exposure to a particular temperature treatment (most often 50^{o}C), we set *RGR* equal to 0. During the course of the experiment, there was a growth chamber malfunction, and so we had to perform experiments with *M. floribundus*, *M. norrisii*, *M. bicolor*, *M. filicaulis*, *M. guttatus*, and *M. laciniatus* at the 35/20^{o}C and the 30/15^{o}C temperature regimes

in a third growth chamber (Percival model PGC-15WC) with the same lighting and identical setup as the original chambers. When using this third chamber, *M. floribundus, M. norrisii*, *M. bicolor*, and *M. filicaulis* were much smaller and looked very unhealthy overall compared to all other growth chamber experiments. Thus, we repeated growth chamber experiments at 35/20^{o}C and the 30/15^{o}C temperature regimes for these species in one of the two original growth chambers in late March through early April of 2013.

Thermal performance breadth and specialist-generalist tradeoffs

We fitted three functions that have been used to describe thermal performance curves to our data: quadratic, Gaussian (Angilletta 2006), and Kumaraswamy (M. Sears, *pers. comm.*) functions using the nlsLM function in the minpack.lm package (Elzhov et al. 2013) in R 3.0.2 (R Core Team 2013). Specifically, we fitted thermal performance curves to family means (mean *RGR* across replicates of each family at each temperature) to avoid pseudoreplication. We then used the Akaike Information Criterion to select the best fitting function for each species pair (Angilletta 2006). We estimated maximum performance (RGR_{max}) as the peak *RGR* value based on the predicted thermal performance curve, optimum temperature for maximum performance (T_{opt}) as the temperature at which RGR_{max} was achieved, and thermal performance breadth as the range of temperatures across which each species achieved ≥ 50% (B_{50}) and ≥ 80% (B_{80}) of its predicted maximum performance (Huey and Stevenson 1979). Our results using B_{50} and B_{80} were qualitatively similar (Table 3.2), so we focus on B_{50} here. Because we fit thermal performance curves to unequal numbers of families for the two species within each pair, species may have narrower thermal performance breadth due to an artifact of having more families. Thus, we fit thermal performance curves to data obtained by randomly sampling an equal number of families per species within each pair 100 times, but results were nearly identical, so we present estimates

of thermal performance curves based on all families here. Because species pair is the unit of replication in this study, we used one-tailed paired Wilcoxon signed rank tests to evaluate the prediction that narrowly distributed species have narrower thermal performance breadths than their widespread relatives, and to detect a specialist-generalist tradeoff between B_{50} and RGR_{max}.

Plasticity

Ideally, to test the hypothesis that broad thermal tolerance is achieved via phenotypic plasticity, we would fit a thermal performance curve to each family mean and test whether families of species with broad thermal tolerance have greater mean thermal performance breadth than families of species with narrow thermal tolerance. Due to lack of sufficient within-family replication, however, we were unable to fit curves to family means. Instead, using family means, we calculated standard deviation in temperature weighted by relative performance, analogous to estimates of niche breadth that weight standard deviation in an environmental axis by relative abundance (Pither and Aarssen 2005), resulting in 9-48 estimates of family-level thermal performance breadth (in units of ^{o}C) per species. Specifically, we used the formula:

$$\sqrt{\sum_{i=1}^{8} p_i (T_i - \bar{T}_w)^2}$$

where p_i corresponds to relative performance (*RGR* at temperature *i* divided by the sum of *RGR* across all 8 temperature regimes, such that the sum of p_i across all temperatures should equal 1); T_i corresponds to the *i*th temperature, and $\bar{T}_w$ corresponds to mean temperature weighted by relative performance (p_i). Because we estimated family-level thermal performance breadth for unequal numbers of families for the two species within each pair, species with more families may have lower average family-level breadth due to an artifact. Thus, we repeated the procedure above by randomly sampling an equal number of families per species within each pair 100 times,

but results were nearly identical, so we present estimates of family-level thermal performance breadth based on all families here. To test the prediction that on average, species with broad thermal tolerance have families with broader thermal tolerance when compared to species narrow thermal tolerance, we used a one-tailed *t*-test for each species pair.

Genetic variation in thermal reaction norms

To test whether widespread species have greater genetic variation in thermal reaction norms than restricted species, we examined the change in performance at extreme temperatures. We focused exclusively on thermal extremes because species did not differ substantially in thermal optima (Table 3.2; Fig. 3.3), suggesting that overall variation in thermal performance breadth between species in each pair resulted primarily from differences in performance at the lowest and highest temperatures. Specifically, using family means for each temperature, we estimated the slope in *RGR* for each family between 15 and 20^{o}C and between 45 and 50^{o}C (Fig. 3.4). As an estimate of genetic variation in thermal reaction norms for each species, we calculated among-family variance across slopes at both temperature extremes: between 15 and 20^{o}C and between 45 and 50^{o}C . Because we estimated among-family variance for unequal numbers of families for the two species within each pair, species with more families may have lower among-family variance due to an artifact. Thus, we repeated estimates of among-family variance by randomly sampling an equal number of families per species within each pair 100 times, but results were nearly identical, so we present estimates of among-family variance based on all families here. We performed one-tailed paired Wilcoxon signed rank tests to assess the hypothesis that species with narrow thermal performance breadth have lower among-family variance in the slope of *RGR* between 15 and 20^{o}C and the slope of *RGR* between 45 and 50^{o}C than species with broad thermal performance breadth.

Climatic variability

To test whether species with ranges encompassing greater variation in temperature should have broader thermal tolerances and larger geographic ranges than species with ranges encompassing less climatic variation, we used standard deviation of mean temperature of warmest quarter (www.worldclim.org) across primary occurrence data of each species to estimate variation in temperature across each species' range. Because our sampling design focused on regionally sympatric populations of species in each species pair, we did not examine temperature seasonality or other measures of thermal variation within each species' sampling site to estimate climatic variability, but we provide such estimates (Wang et al. 2012) to assist in interpretation of results. We used a one-tailed paired Wilcoxon signed rank test to assess support for the prediction that species with broader thermal performance curves should have higher standard deviation in temperature across their known occurrences than species with narrow thermal performance curves.

Results

Thermal performance breadth

A Kumaraswamy function provided the best fit to the thermal performance data of *M. cardinalis*, *M. parishii*, *M. verbenaceus*, and *M. eastwoodiae*; a quadratic function provided the best fit to data of *M. floribundus* and *M. norrisii*; and a Gaussian function provided the best fit to thermal performance data of *M. bicolor*, *M. filicaulis*, *M. guttatus*, *and M. laciniatus* (Tables S2.1 and S2.2 in Appendix 2). Thermal performance breadth (B_{50}) ranged from 12.830^{o}C (*M. laciniatus*) to 32.474^{o}C (*M. verbenaceus*; Table 3.2). Optimum temperature for maximum performance ranged from 24.101^{o}C (*M. floribundus*) to 33.990^{o}C (*M. cardinalis*; Table 3.2).

In four of the five species pairs, the widespread species had a broader thermal performance than the restricted species (Table 3.2; Fig. 3.3), but the magnitude of difference in thermal performance breadth between widespread and restricted species varied among species pairs. Widespread species had marginally significantly larger thermal performance breadths than their narrowly distributed relatives ($W = 14$, $P = 0.0625$), with mean thermal performance breadth of widespread species 2.43^{o}C greater than that of restricted species.

Plasticity

On average, families of *M. verbenaceus*, *M. floribundus*, and *M. guttatus* (species with broader thermal tolerance) exhibited a higher standard deviation in temperature weighted by relative performance than families of *M. eastwoodiae, M. norrisii,* and *M. laciniatus* (species with narrower thermal tolerance), respectively, supporting the prediction that thermal tolerance is achieved via phenotypically plastic families ($t = 4.2181$, df = 41.279, $P < 0.001$; $t = 1.8223$, df = 21.708, $P = 0.04$; $t = 2.02$, df = 12.382, $P = 0.03$, respectively; Table 3.2). Failing to support this prediction, families of *M. parishii* and *M. bicolor* (species with broader thermal tolerance) did not have a higher standard deviation in temperature weighted by relative performance than families of *M. cardinalis* and *M. filicaulis* (species with narrower thermal tolerance), respectively ($t = -0.4843$, df = 39.018, $P = 0.68$; $t = 4.4136$, df = 17.765, $P = 0.34$, respectively; Table 3.2).

Genetic variation in thermal reaction norms

For all species pairs, the species with a broader thermal performance also had significantly greater among-family variance in the slopes of *RGR* between both 15 and 20^{o}C ($W = 15$, $P = 0.03125$) and 45 and 50^{o}C ($W = 15$, $P = 0.03125$; Table 3.2, Fig. 3.4).

Specialist-generalist tradeoffs

Mimulus verbanaceus had a broader thermal performance and a lower maximum *RGR* than its geographically restricted counterpart, *M. eastwoodiae*, thereby supporting the prediction of a specialist-generalist tradeoff between performance breadth and maximum performance (Table 3.2, Fig. 3.3). Within every remaining species pair, however, the species with the broader thermal performance also had a higher maximum *RGR* (Table 3.2, Fig. 3.3). Altogether, species with broader thermal performance curves did not have significantly lower maximum *RGR* than species with narrow thermal performance curves ($W = 14$, $P = 0.9688$; Table 3.2), failing to support the prediction of a specialist-generalist tradeoff between thermal performance breadth and maximum performance.

Climatic variability hypothesis

As predicted, within each species pair, the species whose range encompasses more variation in mean temperature of the warmest quarter also had a significantly broader thermal performance curve ($W = 15$, $P = 0.03125$; Table 3.1). Despite the expectation that widely distributed species may encompass more variation in temperature across their ranges than their narrowly distributed relatives purely by chance (Davies et al. 2009), geographically restricted *M. parishii* had a broader thermal tolerance and experienced more variation in temperature across its range than *M. cardinalis*, its widely distributed counterpart (Tables 3.1, 3.2).

Discussion

In this study, we experimentally quantified thermal performance across 8 temperature regimes for 5,451 plants belonging to 10 species and compared thermal performance breadth, plasticity, quantitative genetic variation, and climatic variability between widespread and restricted species pairs of monkeyflower (Fig. 3.1e). Although four out of five species pairs

supported the hypothesis that species with larger geographic ranges have broader thermal performance than species with small geographic ranges, widespread species as a group only had marginally significantly broader thermal performance than restricted species, suggesting that other niche axes besides temperature may also explain variation in range size among *Mimulus* species. However, the present study builds on our mechanistic understanding of how species acquire broad niches and/or large ranges by demonstrating that both plasticity and genetic variation in thermal performance contribute to broad environmental tolerance. Further, we show that species experiencing greater thermal variation across their ranges have evolved broader thermal tolerances than species with less variation in temperature across their ranges, supporting the climatic variability hypothesis. Below, we discuss these results in light of the natural history of each species and with regard to results from previous studies. In addition, we consider the implications of our results for gaining a better understanding of the relationships between climatic tolerance, geographic range size, extinction risk, and vulnerability to changing climate.

Relationship between thermal performance breadth and geographic range size

In a previous study, we used correlative climatic niche modeling to show that climatic niche breadth is a strong predictor of geographic range size across 72 species of western North American monkeyflower (Sheth et al., in press). Although we controlled for spurious correlations between range size and niche breadth by simulating null geographic distributions (Sheth et al., in press), the present study provides a strong experimental test of the inferences derived from occurrence data and correlative modeling. The present study complements our previous conclusions by showing that in four out of five of the focal species pairs, the widespread species had a broader thermal performance than the geographically restricted species, providing additional support for the niche breadth hypothesis. With the exception of *M.*

cardinalis and *M. parishii*, estimates of thermal performance breadth derived from one population per species (in the present study) for widespread versus restricted species are consistent with estimates of multivariate climatic niche breadth from correlative modeling (Sheth et al., in press).

In the present study, we focused on the thermal niche of *Mimulus* species, based on previous work demonstrating that temperature influences growth and other performance traits in *Mimulus* (Vickery 1967; Vickery 1972; Angert 2006). Apart from differences in thermal tolerance, species in each pair also differ along other abiotic niche dimensions, including habitat and edaphic characteristics. *Mimulus cardinalis* occurs in a variety of moist habitats along seeps, streams, and rivers, while *M. parishii* is restricted to sandy stream edges below 2100 m (Hickman 1993). *Mimulus verbenaceus* occupies desert seeps and creeksides across a broad elevational range, whereas *Mimulus eastwoodiae* occurs in moist, shaded hanging gardens in otherwise arid canyon country (Hiesey et al. 1971; Beardsley et al. 2003). *Mimulus floribundus* inhabits crevices, seeps around granite outcrops, and stream banks, whereas *M. norrisii* grows only in marble crevices (Hickman 1993). *Mimulus bicolor* typically occurs on clay soils while *M. filicaulis* grows on loamy soils, and *M. guttatus* inhabits a diversity of wet places, whereas *M. laciniatus* grows in quick-drying seeps on granite outcrops (Hickman 1993). Despite these and other differences in niche characteristics among species, our current and past work shows that climatic niche properties play an important role in shaping patterns of geographic range size in *Mimulus*. Previous studies of invertebrates (e.g., Calosi et al. 2008; Kellermann et al. 2009; Calosi et al. 2010) and vertebrates (e.g., Cruz et al. 2005) have documented relationships between thermal tolerance and geographic range size, but there have been few tests in plants (but see Luna et al. 2012).

It would be interesting to know whether species with narrow thermal tolerance also specialize along other niche axes such as soil moisture. Similar experiments quantifying performance breadth across a range of soil moistures are currently underway. If species specialize simultaneously along multiple niche axes, then specialization along one niche axis may predict specialization along other niche axes, making specialists particularly vulnerable to extinction risks. Alternatively, specialization along different niche axes may not be correlated (Emery et al. 2012), indicating that different sets of species are predicted to have high extinction risk depending on the niche axis used to assess specialization and the type of environmental perturbation.

Support for the niche breadth hypothesis may be equivocal if tests are limited to a single population that does not represent the niche breadth of the entire species. Despite the many studies that have found support for the niche breadth hypothesis (Pyron 1999; Brandle et al. 2003; Hurlbert and White 2007; Köckemann et al. 2009; Verberk et al. 2010; Emery et al. 2012), few have addressed the potential for local adaptation to facilitate range expansion. Thus, a major question that remains unanswered is whether widespread species have achieved large distributions by means of local adaptation to a variety of environments, or because individuals across the species' range have general-purpose genotypes that permit broad environmental tolerances (Baker 1965). To distinguish the latter from the former, niche breadth must be quantified for multiple populations per species. Examination of niche breadth across multiple populations would allow one to assess how a species' total niche is partitioned among populations and families or individuals (in the case of clonal species). Thus, assessing the extent to which species accumulate niche breadth through populations that are locally adapted to different environments, or by having populations with broad environmental tolerances across the

range would yield important insights about the evolution of niche breadth and range size (Slatyer et al. 2013). In a study quantifying thermal tolerance for multiple populations across the latitudinal range of a widespread copepod, species-level thermal tolerance was far greater than thermal tolerance for any given population (Kelly et al. 2012), highlighting the value of quantifying thermal performance across several populations per species.

Even with our conservative approach of estimating thermal tolerance for only one population per species, we captured variation in thermal tolerance among *Mimulus* species, suggesting that innate differences in thermal tolerance may contribute to variation in geographic range size among species. Despite being more widespread, *M. cardinalis* may have a narrower thermal performance breadth than *M. parishii* because species-level niche breadth of *M. cardinalis* is achieved via locally adapted populations differing in thermal optima for performance. A study of variation in thermal performance among populations of *M. cardinalis* reveals that populations within the northern half of the species' range have overlapping but variable thermal optima for performance (Angert et al. 2011), and thermal optimum would likely vary to a greater degree if populations from the southern half of the range were also included. If more populations were considered, the magnitude of difference in thermal tolerance between widespread and restricted species would probably be even greater since widespread species by definition encompass a broader latitudinal range, and among-population variation would likely lead to even broader thermal tolerance.

The present study adds to a growing number of empirical tests of the niche breadth hypothesis involving comparisons of niche breadth between widespread and restricted congeners. For example, studies of two clades of diving beetles report a positive relationship between thermal tolerance and latitudinal extent, highlighting the benefits of experimentally and

phylogenetically controlled tests of the niche breadth hypothesis (Calosi et al. 2008; Calosi et al. 2010). Here, we expand upon such comparative studies by further examining the mechanisms that may promote or constrain the evolution of broad thermal tolerance.

Plasticity and genetic variation in thermal performance

We document evidence that both plasticity and genetic variation in thermal performance contribute to an overall broad thermal tolerance. These findings provide additional insights to studies showing that species with broad geographic distributions have greater intraspecific variation in traits but that have not quantified the roles of plasticity and heritable variation in shaping species'-level niche breadth (e.g., Sides et al. 2014). In three species pairs, the species with broader thermal tolerance consisted of more thermally tolerant families than species with narrow thermal tolerance, highlighting the role of within-family plasticity in determining species-level thermal tolerance. Species with broader thermal tolerance had greater genetic variation in thermal performance at both low and high temperatures than those with narrower thermal tolerance, suggesting that genetic variation in ecologically relevant traits may facilitate the evolution of broad climatic tolerances. This finding is consistent with a recent study of thermal tolerance and species' distributions of *Drosophila* (Kellermann et al. 2009). However, genetic variation for thermal performance may vary across species' ranges, and theoretical and empirical work suggests that populations at the edges of species' ranges may lack genetic variation in one or more ecologically important traits (Antonovics 1976; Pujol and Pannell 2008). Thus, it would be useful to estimate genetic variation in relevant traits across species' ranges to further understand how genetic variation may promote niche evolution and range expansion.

Specialist-generalist tradeoffs

Consistent with a growing body of literature suggesting that a "jack of all temperatures" can be a "master of all" (reviewed in Angilletta 2009), our results do not provide strong support for the notion that specialist-generalist tradeoffs constrain the evolution of broad environmental tolerance. We only found evidence of a tradeoff between thermal performance breadth and maximum performance for *M. verbenaceus* and *M. eastwoodiae,* the species pair with the largest difference in thermal performance breadth (Figure 3.3, Table 3.2). Instead, within every remaining species pair, the species with the broader thermal performance also had a higher maximum *RGR* (Fig. 3.3, Table 3.2), supporting the idea that "broader is better." *Mimulus guttatus* and *M. laciniatus* were the only species pair supporting the ideas that "hotter is better" (Hamilton 1973; Huey and Kingsolver 1989) and "hotter is broader" (Knies et al. 2009), based on *M. guttatus* having a higher T_{opt}, a broader thermal performance curve, and a higher maximum *RGR* than *M. laciniatus*. Given that the pair with the greatest difference in thermal performance breadth was the only species pair exhibiting a tradeoff between breadth and maximum performance, differences in breadth among species may need to be substantial to detect a cost in maximum performance. Although we did not often detect costs in maximum *RGR* at the expense of having a broad performance curve, there could be costs in other performance metrics. For example, rapid growth may result in lower seed production, but we were unable to detect such tradeoffs because we only measured *RGR*.

Climate variability hypothesis

As predicted by the climate variability hypothesis, our results are consistent with the idea that species experiencing greater variation in climate have evolved broader climatic tolerances than species originating from more climatically stable areas. In particular, our finding that

thermal tolerance estimated from one population per species was related to thermal variation across a species' range for all five species pairs suggests that the climatic variability hypothesis may explain variation in climatic tolerances even at smaller spatial scales that do not encompass temperate-tropical latitudinal gradients. Although *M. cardinalis* has a much larger geographic range than *M. parishii* (Fig. 3.2), *M. parishii* still had both a broader thermal tolerance and greater thermal variation across its geographic range. Consistent with our results, previous work suggests that temperate trees have broader thermal performance curves than tropical species when measuring performance as photosynthetic rate (Cunningham and Read 2002). Further, previous literature suggests that plant species are limited by reduced performance at low temperatures (Woodward et al. 1990; Cunningham and Read 2002; Pither 2003), yet we do not find clear evidence that suggests that narrow thermal tolerance results from poor performance at low (rather than high) temperatures. Specifically, *M. verbenaceus* and *M. guttatus* (species with broader thermal tolerance) were more tolerant to high temperatures than *M. eastwoodiae and M. laciniatus* (species with narrower thermal tolerance), respectively; *M. parishii* (species with broader thermal tolerance) was more tolerant to low temperatures than *M. cardinalis* (species with narrower thermal tolerance), and *M. floribundus* and *M. bicolor* (species with broader thermal tolerance) were more tolerant to both low and high temperatures than *M. norrisii* and *M. filicaulis* (species with narrower thermal tolerance), respectively (Fig. 3.3). Though we quantified thermal tolerance for small seedlings in the case of *M. cardinalis*, *M. verbenaceus*, and *M. eastwoodiae*, it is possible that survival and fecundity late in the growing season might be more important for explaining differences in distribution among perennial species with long growing seasons and the need to survive over winter.

Unlike many other tests of the climatic variability hypothesis, our work sheds light on the mechanisms by which climatic variability may lead to broad environmental tolerance. First, in all five species pairs, the species with broader environmental tolerance and greater variability in climate across its range also exhibited more quantitative genetic variation than the species with narrower thermal tolerance and less climatic variability across its range. Second, in three species pairs, the species with broader thermal tolerance and greater variability in temperature across its range also displayed a greater level of plasticity than the species with narrower thermal tolerance and less variation in temperature across its range. Together, these findings indicate that the effects of climatic variability on species-level environmental tolerance may be mediated by quantitative genetic variation and phenotypic plasticity.

Caveats

When interpreting results of our study, there are several caveats that should be considered. First, we only included one population per species, thereby ignoring the effects of locally adapted populations and intraspecific variation on species-level thermal performance breadth. Including only one population per species likely leads to an underestimate of species-level thermal performance breadth, but the breadths of widespread species should be more severely underestimated than that those of restricted species. Thus, we emphasize that such a study design is conservative with respect to the niche breadth hypothesis. Further, some species were sampled at their latitudinal range centers while others were sampled near a northern or southern range edge (Fig. 3.2). Such idiosyncratic sampling could potentially affect the observed patterns of genetic variation and plasticity. Second, due to logistical benefits and the potential effects of early stage performance on the probability of survival to flowering, we estimated performance as *RGR*. However, other performance metrics such as fecundity would provide

further insights into understanding fitness tradeoffs among species that differ in thermal tolerance. Third, we used simple thermal regimes that did not incorporate daily fluctuations in temperature that plants experience in natural settings. Finally, we caution that our conclusions are based on only five species pairs, and studies quantifying thermal performance for a greater number of populations and species are needed.

Other drivers of variation in thermal tolerance and/or geographic range size

Although our focal species within each pair are closely related and should be of similar age, they differ in several other characteristics aside from ecological niche dimensions that could contribute to variation in range size. For example, populations of *M. cardinalis*, *M. verbenaceus*, *M. eastwoodiae*, *M. bicolor*, *M. filicaulis*, and *M. guttatus* included in our study were predominantly outcrossing, whereas populations of *M. parishii*, *M. floribundus*, *M. norrisii*, and *M. laciniatus* were predominantly selfing (S. Sheth, unpubl. data). Mating system should affect a species' ability to colonize novel locations and environments (Baker 1955). On the one hand, selfing could facilitate range expansion when compared to outcrossing, which relies on pollinator availability, yielding the expectation that selfing species should have larger geographic ranges than closely related outcrossing species (Henslow 1879; Baker 1955). On the other hand, selfing would alter the distribution of genetic variation within and among populations, and the reduction of genetic variation associated with selfing could inhibit the evolution of broad environmental tolerance (Lowry and Lester 2006), confounding our understanding of relationships between mating system and range size. Although we did not design our study to test the role of selfing vs. outcrossing in explaining variation in range size among species, our results do not support the notion that selfing affects thermal tolerance and/or range size. We had two species pairs in which one species is predominantly selfing and the other is not, and in one case the selfing species (*M.*

parishii) has a broader thermal tolerance yet a smaller range than the outcrossing species (*M. cardinalis*), and in the other case the selfing species (*M. laciniatus*) has a narrower thermal tolerance and a smaller range than the outcrossing species (*M. guttatus*).

Conclusions

In this study, we demonstrate that environmental tolerance is shaped by both intrinsic factors such as plasticity and genetic variation in ecologically relevant traits, and extrinsic factors such as variation in selection pressures across geography. Inherent species-level differences in environmental tolerance, in turn, can lead to variation in geographic range size among species. We stress the need to collect more extensive physiological data on environmental tolerances of a greater number of species and populations if we are to draw broader conclusions about the mechanisms shaping patterns of environmental tolerance and geographic range size. Our results have important implications for species with narrow thermal tolerance, which may be particularly vulnerable to climatic changes, through both narrow thermal tolerance itself and because they may lack sufficient phenotypic plasticity to cope with altered temperature regimes or genetic variation to respond to novel selection pressures. In contrast, species currently experiencing high variation in temperature across their ranges may be buffered against extinction related to climatic changes because they have evolved tolerance to a broad range of temperatures. Given projected increases in temperatures of ~2-5^{o}C by 2099 in North America according to a medium-level emissions scenario (Meehl et al. 2007), even small differences in thermal tolerance among species could translate into important differential responses to changing climate.

Table 3.1 Widespread and restricted species pairs (denoted by species with the same letter subscript) used in this study. Range size: area of minimum convex polygon encompassing primary occurrence data; LM: latitudinal midpoint of primary occurrence data; LR: latitudinal range: difference between maximum and minimum latitudes encompassed by primary occurrence data; $Mean_{ts}$ and SD_{ts}: mean and standard deviation in mean summer temperature (from 1970-2012; Wang et al. 2012) of sampled populations included in study (Fig. 3.2); $Mean_{tw}$ and SD_{tw}: mean and standard deviation in mean temperature of warmest quarter (www.worldclim.org) across primary occurrence data. *See Sheth et al. (in revision) for details.

Species	Range size* (km^2)	LM* (^{o})	LR* (^{o})	$Mean_{ts}$ (^{o}C)	SD_{ts} (^{o}C)	$Mean_{tw}$* (^{o}C)	SD_{tw}* (^{o}C)
M. cardinalis[a]	470,772	36.1	15.8	26.80	0.78	20.28	2.87
M. parishii[a]	95,116	33.7	4.4	27.41	0.78	22.49	3.46
M. verbenaceus[b]	514,264	29.9	14.8	22.30	0.87	23.38	3.84
M. eastwoodiae[b]	43,862	37.4	2.6	21.91	0.78	22.66	2.06
M. floribundus[c]	4,423,834	36.7	34.6	24.05	0.86	20.99	4.33
M. norrisii[c]	275	36.4	0.24	25.58	0.78	22.33	1.85
M. bicolor[d]	56,551	38.5	4.8	18.81	0.95	19.99	2.43
M. filicaulis[d]	436	37.8	0.40	20.89	1.05	18.99	1.28
M. guttatus[e]	12,053,145	41.1	47.7	20.89	1.05	19.85	5.20
M. laciniatus[e]	25,048	38.2	3.3	20.89	1.05	17.35	4.22

Table 3.2 Widespread (w) and restricted (r) species pairs (denoted by species with the same letter superscript) used in this study. B_{50}: thermal performance breadth based on relative growth rate (*RGR*); T_{opt}: optimum temperature for *RGR*; RGR_{max}: maximum relative growth rate; $\overline{WSD}$: standard deviation in temperature weighted by relative performance, representing an estimate of family-level thermal performance breadth; V_{cold}: among-family variance in the slope of *RGR* from 15 to 20°C; V_{hot}: among-family variance in the slope of *RGR* from 45 to 50°C; *N*: number of families planted per temperature. For the first three species pairs below, the units of *RGR* are based on leaf counts (leaf leaf^{-1} day^{-1}), and for the remaining two species pairs the units of *RGR* are based on stem length (cm cm^{-1} day^{-1}).

Species	B_{50} (°C)	B_{80} (°C)	T_{opt} (°C)	RGR_{max}	$\overline{WSD}$ (SE)	V_{cold}	V_{hot}	*N*
M. cardinalis[a] (w)	25.916	14.712	33.916	0.052	9.17 (0.25)	0.000017	0.000021	22
M. parishii[a] (r)	26.632	15.881	31.313	0.085	9.02 (0.16)	0.000080	0.000082	50
M. verbenaceus[b] (w)	32.474	23.638	24.582	0.029	8.98 (0.25)	0.000021	0.000015	24
M. eastwoodiae[b] (r)	23.142	13.197	24.323	0.048	7.74 (0.16)	0.000017	0.000010	42
M. floribundus[c] (w)	26.982	17.064	24.101	0.120	8.55 (0.27)	0.000153	0.000175	18
M. norrisii[c] (r)	25.956	16.416	24.496	0.077	7.58 (0.46)	0.000064	0.000026	18
M. bicolor[d] (w)	20.185	11.453	27.334	0.225	7.81 (0.23)	0.000100	0.000769	23
M. filicaulis[d] (r)	19.086	10.829	28.41	0.128	7.65 (0.32)	0.000015	0.000113	13
M. guttatus[e] (w)	14.230	8.073	32.223	0.442	8.19 (0.47)	0.000045	0.003926	11
M. laciniatus[e] (r)	12.830	7.28	30.341	0.350	7.18 (0.16)	0.000027	0.000302	14

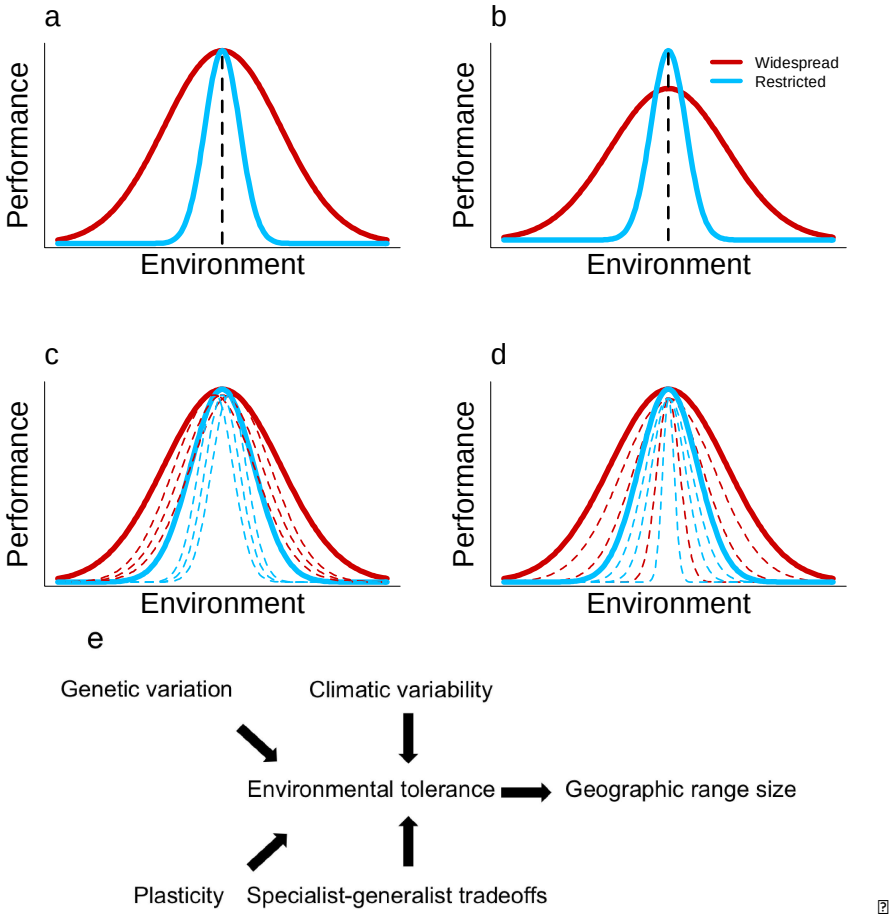

Figure 3.1 Hypothesized relationship between environmental tolerance and geographic range size for two closely related species in the absence (a) and presence (b) of a specialist-generalist tradeoff between width of performance curve and maximum performance. Dashed lines in panels a and b indicate optimum environment for maximum performance. (c) Illustration of how phenotypically plastic genotypes or families (i.e., genotypes or families that are able to maintain high performance across a broad range of environments; represented by dashed curves) can lead to a broad species-level environmental tolerance (solid curves) (d) Illustration of how genetic variation in environmental tolerance among genotypes or families (dashed curves) can result in broad species-level environmental tolerance (solid curves). In panels a-d, the geographically widespread species has a broader environmental tolerance than the geographically restricted species. (e) Conceptual diagram illustrating how climatic variability, plasticity, genetic variation in environmental tolerance, and specialist-generalist tradeoffs are hypothesized to affect geographic range size via their effects on environmental tolerance. Though there are other potential relationships among the variables depicted (e.g., climatic variability may increase genetic variation and/or lead to specialist-generalist tradeoffs), only paths that represent predictions tested in this study are depicted here. Arrows represent positive effects. In Tables 3.1 and 3.2, B_{50} corresponds to environmental tolerance, SD_{tw} corresponds to climatic variability, specialist-generalist tradeoffs occur if the species in each pair with a greater B_{50} has a lower RGR_{max}, $\overline{NT_{fam}}$ refers to within-family plasticity, and V_{cold} and V_{hot} are estimates of genetic variation at the cold and hot extremes of the thermal reaction norm, respectively.

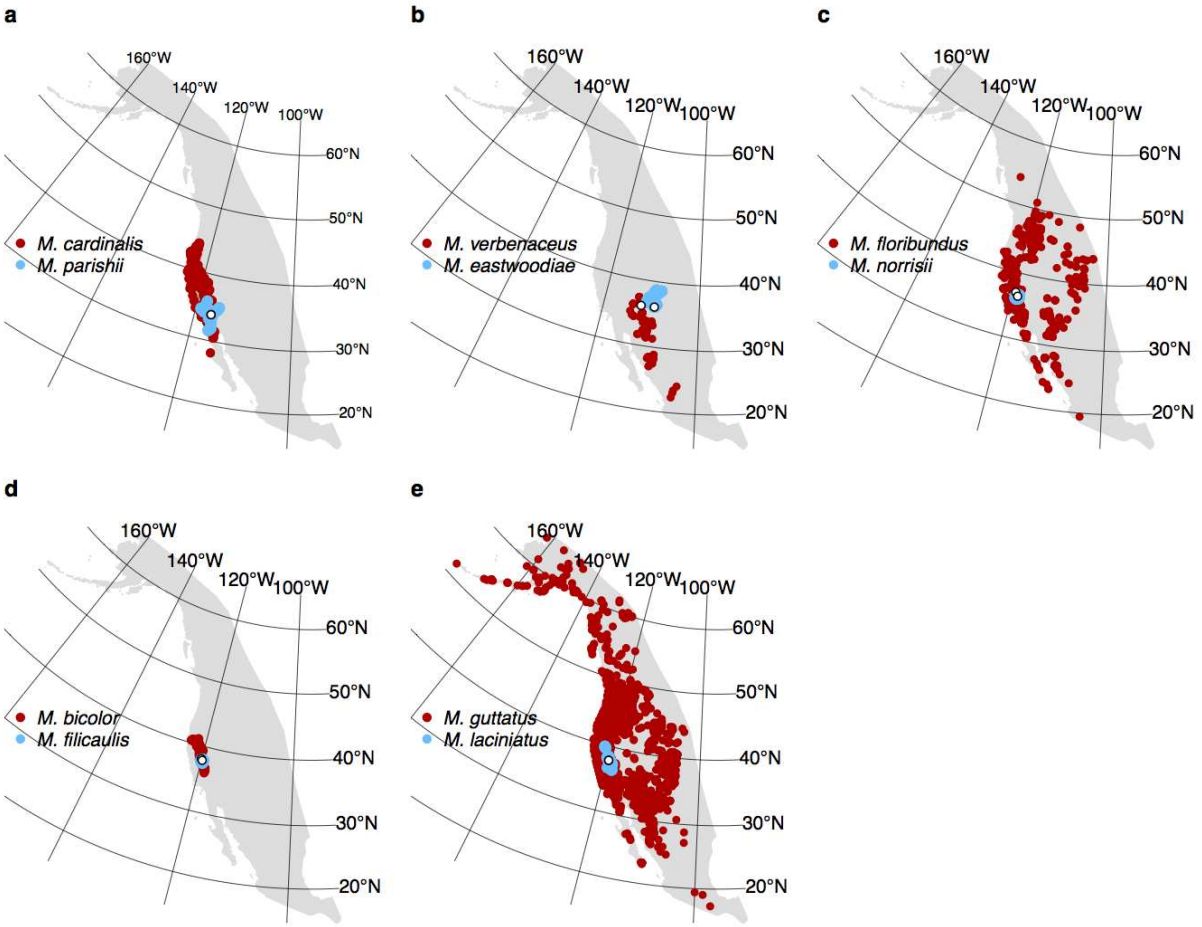

Figure 3.2 Herbarium specimen localities with red corresponding to the widespread species and blue corresponding to the restricted species in each pair, and sampling localities for each species are shown with white circles. In panels c and d we sampled seed for species within each pair from different sites, but sampling localities are so close together that they overlap at the scale shown.

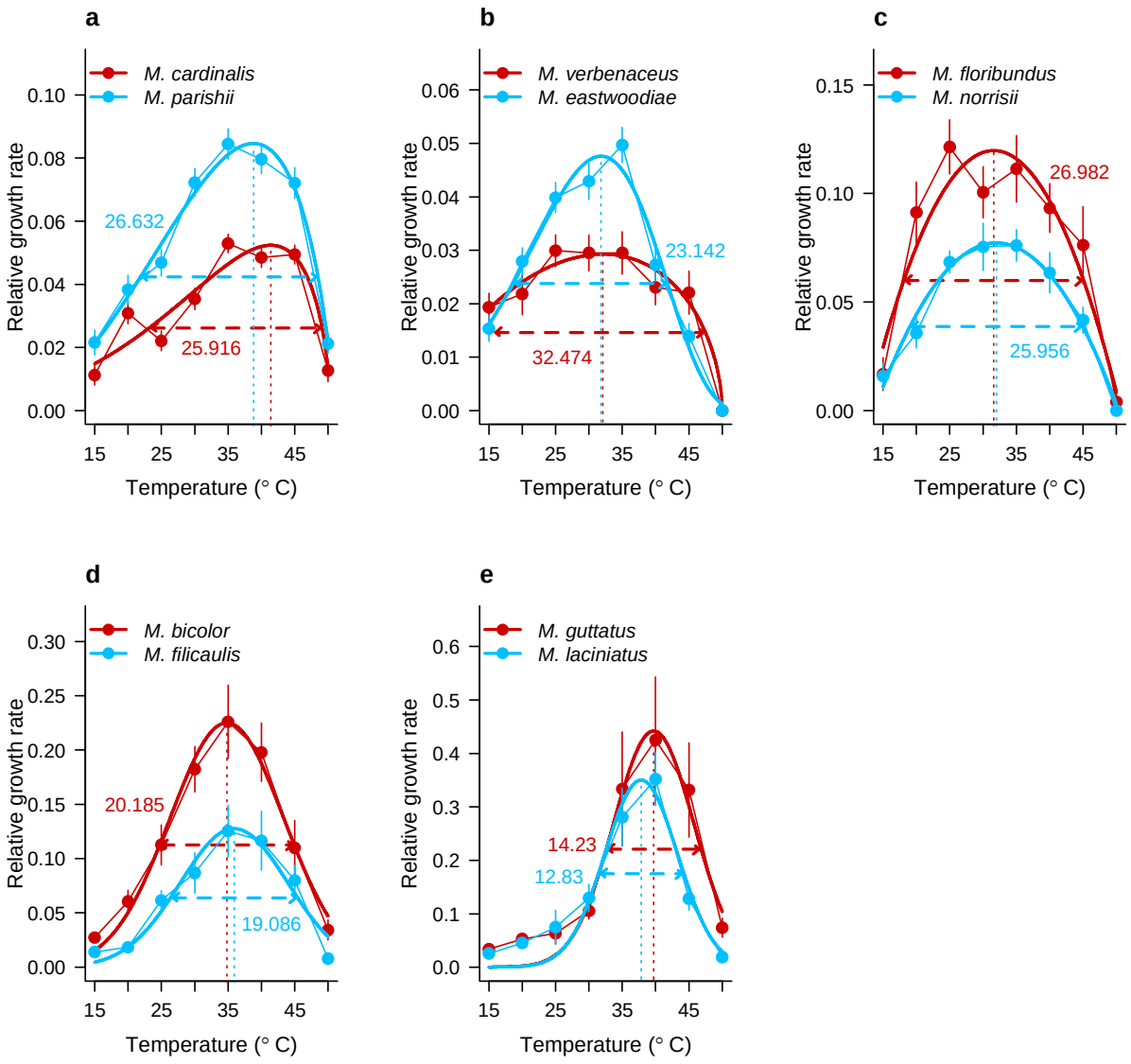

Figure 3.3 Observed (± 1 SE) and fitted thermal performance curves for each species, with red corresponding to the widespread species and blue corresponding to the restricted species in each pair. Horizontal lines and numerical values represent thermal performance breadth (^{o}C), and vertical lines represent optimum temperature for maximum performance (^{o}C). In panels a and b, Kumaraswamy functions were fit to relative growth rate in leaf number; in panel c, quadratic functions were fit to relative growth rate in leaf number (with units as number number^{-1} day^{-1}); and in panels d and e, Gaussian functions were fit to relative growth rate in stem length (with units as cm cm^{-1} day^{-1}). X-axes represent diurnal temperatures used in experiments. With the exception of *M. cardinalis* and *M. parishii* (panel a), within each species pair, the widespread species had a broader thermal performance curve than the restricted species.

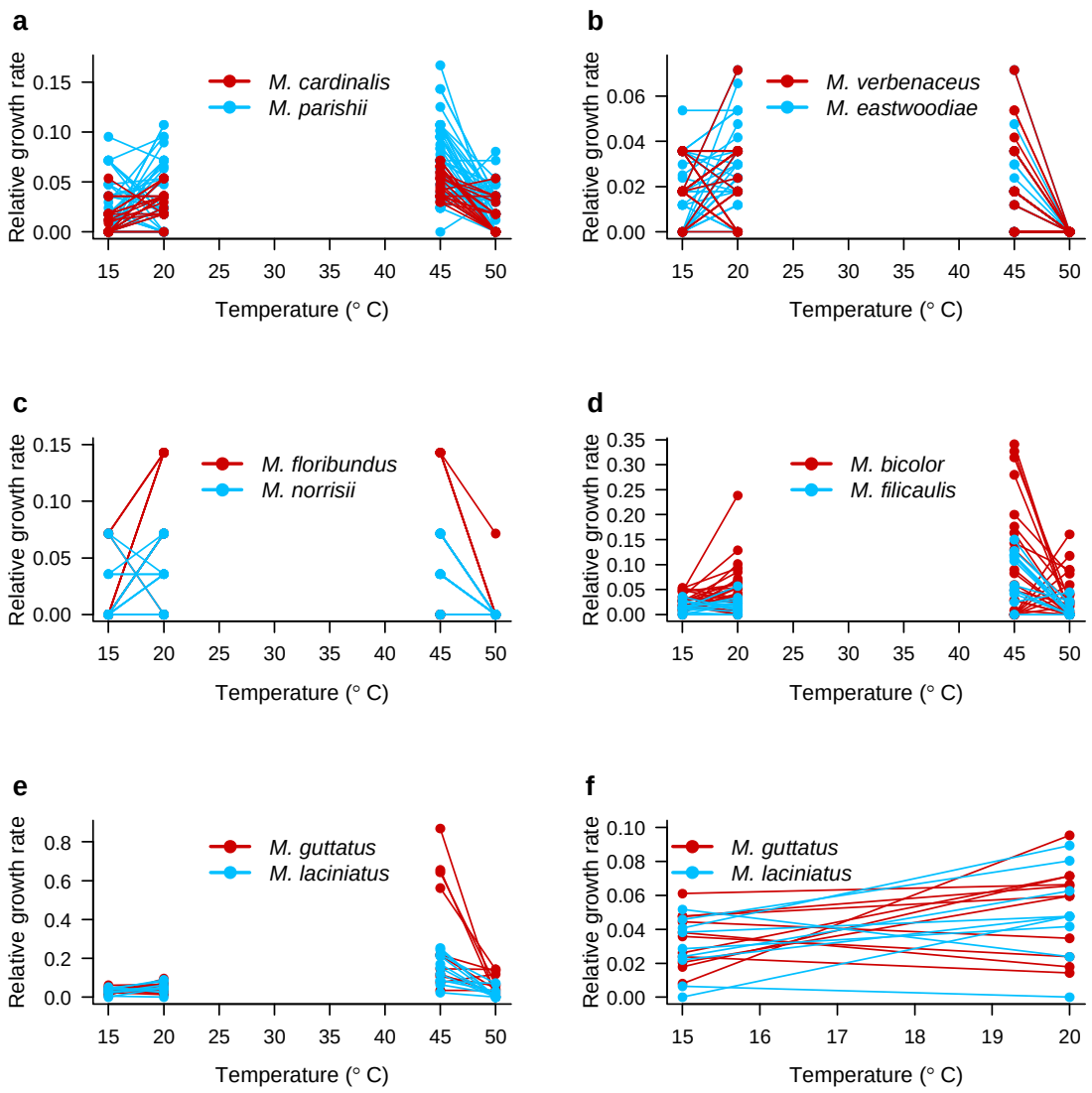

Figure 3.4 Slopes connecting family means of relative growth rate between 15 and 20^{o}C and between 45 and 50^{o}C for each species, with red corresponding to the widespread species and blue corresponding to the restricted species in each pair. To estimate genetic variation in thermal performance at low and high temperatures, we calculated among-family variance across slopes of each species at each temperature extreme. Panel f shows slopes in relative growth rate between 15 and 20^{o}C for *M. guttatus* and *M. laciniatus* in more detail. In panels a-c, relative growth rate in leaf number is in units of number number^{-1} day-^{1}, and in panels d-f, relative growth rate in stem length has units of cm cm^{-1} day^{-1}. Species that appear to have a small number of families actually have multiple families with overlapping values of relative growth rate at each temperature.

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4. EVOLUTIONARY POTENTIAL DOES NOT LIMIT RANGE EXPANSION IN THE SCARLET MONKEYFLOWER

Summary

Every species has a limited geographic range, leading to the question of what prevents a species from continually expanding its range by adapting to conditions at the range edge. One hypothesis that explains evolutionary stasis at range limits is that populations at the edge of a species' range lack sufficient genetic variation in ecologically important traits to respond to natural selection. This hypothesis predicts that populations near the range margin should exhibit lower responses to selection than populations near the range center. To test this prediction, we performed artificial selection experiments using the scarlet monkeyflower (*Mimulus cardinalis*), a perennial herb that spans a broad latitudinal range in western North America, to compare genetic variation in flowering time among populations at the latitudinal center, northern edge, and southern edge of the species' range. Contrary to prediction, we found that southern populations exhibit significantly greater response to selection on flowering time than central or northern populations. While response to selection on flowering time varied among populations across the range in our study, high and low latitude edges exhibited drastically different magnitudes of response to selection, and the patterns we observed cannot be clearly explained by range position alone. These results provide critical insights about how spatial variation in adaptive potential may affect population persistence in the face of climate change.

Introduction

Although species vary in geographic range size, every species has a limited geographic range (Gaston 2003), leading to the question of what prevents a species from continually

expanding its range by broadening its niche. A species' range has been regarded as the ecological niche projected onto geography (Pulliam 2000), such that range limits coincide with the species' niche boundaries. Although some species' ranges are not at equilibrium with current environments (Svenning and Skov 2004), many species show reduced fitness and negative population growth beyond current range boundaries (reviewed in Sexton et al. 2009). Concordance between range and niche limits indicates constraints on adaptation to novel conditions beyond the range (Antonovics 1976). Investigations of the evolutionary processes operating in populations at the edges of species' ranges inform our understanding of the causes of stable range limits. Populations occupying the edges of species' geographic distributions may suffer a greater risk of extinction than populations at the range center (Hardie and Hutchings 2010), highlighting the importance of marginal populations to the persistence of species in the face of changing climate (Arndt and Rémy 2005). Populations at the leading edge of a species' distribution may benefit either from "preadaptations" resulting from gene flow from central populations or from the ability to track favorable climate through migration, whereas populations at the trailing edge may need to adapt to novel conditions to persist (Jump and Peñuelas 2005). Consequently, investigations of whether populations at the peripheries of species' geographic ranges can adapt to marginal environments are crucial to understanding how ranges may respond to environmental change.

Several classes of hypotheses have been proposed to explain evolutionary constraints to range expansion. First, asymmetrical gene flow from large, locally adapted central populations at the range center might introduce maladaptive alleles to populations at range margins (Haldane 1956; Kirkpatrick and Barton 1997). This effect is intensified when competitive interactions among species are taken into account (Case and Taper 2000; Case et al. 2005), and researchers

have shown that, even in the absence of gene flow, selection and competition for resources can lead to evolutionarily stable range limits (Price and Kirkpatrick 2009). Another hypothesis that has been proposed to explain how parapatric range limits arise considers the role of hybridization in hindering adaptation at range edges (Goldberg and Lande 2007). Here, we focus on a third hypothesis, that populations at range margins lack sufficient genetic variation to respond to natural selection, thereby leading to evolutionarily stable range limits (Antonovics 1976). Despite extensive theoretical examination of these hypotheses, empirical tests remain scarce.

Adaptation by natural selection depends on genetic variation, the raw material for evolutionary change. The magnitude of genetic variation in traits under natural selection may affect species' abilities to expand their ranges via local adaptation to conditions at and beyond range margins. Populations at range margins may exhibit lower genetic variation in ecologically important traits for a number of reasons. First, edge populations are often small and/or isolated (Eckert et al. 2008), thus potentially lacking genetic variation as a result of drift, founder events, genetic bottlenecks, and/or inbreeding associated with small population size. The expectation that marginal populations are smaller than central ones stems from the abundant center hypothesis (Brown et al. 1995), which predicts that habitat suitability decreases from the center to the edges of species' ranges, in turn leading to a reduction in abundance from the range center towards the margins. Second, strong directional selection at range edges may lead to the fixation of favored alleles, thereby exhausting genetic variation in range-limiting traits (Blows and Hoffmann 2005). Although patterns of gene flow from central to edge populations could influence predictions about patterns of quantitative genetic variation across the range, it is unclear how asymmetrical gene flow from the range center to edge would affect additive genetic variance in edge populations (Case and Taper 2000). For example, if edge populations are small

and characterized by low levels of genetic variation, then gene flow from the center may provide genetic rescue (Holt and Gomulkiewicz 1997; Barton 2001; Sexton et al. 2011). On the other hand, if gene flow from the range center introduces maladaptive alleles in edge populations, edge populations may decline in size, leading to a decrease in genetic variation in marginal populations (Mayr 1963). Regardless of the interplay between gene flow and quantitative genetic variation, if range expansion via local adaptation is constrained due to a lack of genetic variation in ecologically relevant traits, then response to selection on such traits should be lower at range edges than at the center of a species' range (Blows and Hoffmann 2005). Most studies comparing genetic variation between central and marginal populations within a species' range have focused on neutral genetic variation, showing that genetic variation decreases and genetic differentiation increases from the center to the margins of species' ranges (Eckert et al. 2008; Hardie and Hutchings 2010). Yet, differences in neutral genetic diversity between central and peripheral populations are relatively minute (Eckert et al. 2008), and additive genetic variance, the amount of phenotypic variation attributable to additive genetic effects, is required for evolution by natural selection. However, few studies have compared additive genetic variance in ecologically important traits between the range center and edges (but see Pujol and Pannell 2008; Kelly et al. 2012). In this study, we used artificial selection, a statistically powerful approach to quantify adaptive potential (Conner 2003), to compare adaptive potential among six populations across the geographic range of the perennial herb *Mimulus cardinalis* (Phrymaceae). Specifically, we tested whether populations from the northern and southern range edges had a lower response to selection on flowering time than populations from the range center.

Methods

Study system and sampling

To determine whether genetic variation constrains local adaptation at range margins, we compared genetic variation in ecologically important traits among populations at the center and latitudinal edges of the geographic distribution of the scarlet monkeyflower, *Mimulus cardinalis* (recently renamed *Erythranthe cardinalis*; Nesom 2014). A perennial herb with red flowers characteristic of hummingbird pollination, *M. cardinalis* is broadly distributed along seeps and streamsides from sea level to 2400 m from southern Oregon to northern Baja California and from the Pacific coast east to the Sierra Nevadas (Fig. 4.1a; Hickman 1993). *Mimulus cardinalis* is an appropriate system for testing hypotheses about range limits because it is the subject of several past and ongoing studies of ecological and evolutionary determinants of range limits. In particular, fitness, demographic, and physiological constraints to local adaptation at altitudinal range limits have been thoroughly investigated (Angert and Schemske 2005; Angert 2006a, b; Angert et al. 2008; Angert 2009), providing key baseline information about hypothesized processes that give rise to range limits and potential traits under natural selection. Further, investigations of the role of gene flow, demographic history, and contemporary metapopulation and demographic processes across the latitudinal range of *M. cardinalis* are underway (e.g., Paul et al. 2011). Thus, our investigation of adaptive potential complements ongoing studies testing other hypotheses to explain latitudinal range limits in *M. cardinalis*. In Fall 2010, we collected seeds from 80-200 individuals in each of two northern edge (hereafter referred to as "N1" and "N2"), two southern edge (hereafter referred to as "S1" and "S2"), and two latitudinally central populations (hereafter referred to as "C1" and "C2") across the geographic range of *M. cardinalis* (Table 4.1; Fig. 4.1a). Herbarium specimens from northern Baja California are of

questionable identity and are morphologically more similar to *M. verbenaceus* (S. Sheth, *pers. obs.*), so populations sampled near the southern range margin represent the southernmost populations that can be definitively identified as *M. cardinalis*.

Artificial selection experiments

We quantified genetic variation in one phenotypic trait that likely influences survival and reproduction under different temperature and precipitation regimes associated with different latitudinal range positions (Table 4.1; Fig. 4.1a; Wang et al. 2012) and that can be measured in a non-destructive manner. Latitudinal variation in flowering time, measured as the number of days from germination to first flower, should comprise a key adaptation to local environmental cues for flowering plants (Matsuoka et al. 2008). Moreover, previous work has documented rapid responses to artificial selection on flowering time (Burgess et al. 2007), suggesting that flowering time is a heritable trait that could potentially shift in response to only a few generations of selection. We carried out artificial selection experiments on flowering time to quantify response to selection (R) as a direct measure of a population's adaptive potential. Further, R is proportional to additive genetic variance (V_A), defined as the amount of phenotypic variance that is due to additive genetic causes, based on the breeder's equation (Conner 2003). In December 2010, we planted field-collected seeds from each population in the Colorado State University Greenhouse (Fig. 4.1b). In Spring 2011, we randomly crossed individuals within each population to produce outcrossed seeds, resulting in 40–101 full-sibling families per population (Fig. 4.1b). In January 2012, we filled 3" pots with Farfard 4P Mix potting soil with a thin layer of Farfard Superfine Germinating mix on top (Conrad Farfard, Inc., Agawam, MA, USA), and we planted 3 seeds per family per pot. Each pot was misted daily until seedlings became established. During this time, we scored germination time for each seedling daily. Three weeks

after planting, we thinned each pot down to one randomly chosen seedling, resulting in one individual from each full-sibling family in each population on which we measured flowering time. To create selection lines for flowering time, we randomly selected 25% of the individuals from each population for a control line, and we selected individuals that fell into the earliest 25% of flowering time for an early flowering selection line and individuals that fell in the latest 25% of flowering time for a late flowering selection line (Fig. 4.1b). We then randomly crossed individuals in pairs within each of the resulting 18 lines (1 early flowering, 1 control, and 1 late flowering line for each of 6 populations), maintaining a population size of 40–104 individuals per selection line (for a total of 1,368 individuals planted across all populations in each generation) in each generation while avoiding crosses between close relatives. For each randomly paired set of individuals in a given cross, each parent served as a pollen donor and a pollen recipient once. To maintain equal population sizes from one generation to the next, we planted 4 replicates of each cross type, resulting in 8 replicates for each full-sibling family. We repeated this selection process for two generations (once from January through May 2013 and again from January through May 2014; Fig. 4.1b) and measured *R*, which is the change in the mean of the selected phenotypic trait across each generation, and the selection differential (*S*), the difference between the mean phenotypic value of the entire population and mean of the subset selected for breeding in each generation (Conner 2003). In the first generation after selection (2013), of the 1,368 pots into which we planted, only 16 did not yield plants that survived to flowering, and in the second generation after selection (2014), only 21 pots did not have plants that survived to flower, thus yielding a balanced design in which the number of individuals in each selection line of a given population was very similar. To account for positional effects in the greenhouse, within each selection line in each population, individuals in

pots were arranged randomly among trays (16 pots/tray). Randomization was performed within each selection line and population to prevent competition among plants that drastically differed in size. Trays were then randomized on the greenhouse benches and rotated weekly. Rather than replicating upper and lower selection lines for each population and trait, which was logistically infeasible if also replicating for the main effect of interest (regions), the units of replication were populations within regions. All generations were grown in the Colorado State University Greenhouse with a 16 h day/8 h night photoperiod with day temperature programmed to ~25^{o}C and night temperature at ~20^{o}C.

Within-population tests for significant response to selection

At the end of the artificial selection experiment, we computed a cumulative *S* and *R* for each selection line across two generations. To account for uneven contributions of families to each generation of artificial selection, we weighted selection differentials according to the proportionate contributions of parents (Falconer and Mackay 1996). We did not adjust trait values by control means when computing *S* because we assumed that the environment remained constant within each generation (B. Walsh, pers. comm.). We estimated cumulative *S* as the sum of weighted *S* from the first and second generations of selection. To test for significant responses to selection within each population after two generations of selection, we modeled flowering time in the final generation (2014) as a function of selection line (early flowering, control, or late flowering) as a fixed effect and family as a random effect, and used Type III tests to assess the significance of fixed effects using the Satterthwaite approximation for denominator degrees of freedom. For populations in which there was a significant effect of selection line on flowering time, we examined pairwise differences in mean flowering time among selection lines using post-hoc Tukey HSD tests. Subsequently, we estimated cumulative *R* for each early or late

flowering line in each population as the difference between the least square mean of the early or late flowering line and the least square mean of the control line in the final generation (2014) to account for potential changes in phenotype caused by environmental variation among generations (Falconer and Mackay 1996).

Analyses comparing responses to selection among central and marginal populations

To test whether response to selection on early or late flowering was lower at the northern and southern range edge than at the range center, we used a linear mixed model with region, selection line (early and late flowering), and their interaction as fixed effects, and population and family as random effects. Analogous to the within-population analyses described above, we first standardized flowering time in the final generation (2014) by subtracting the mean flowering time of the control line of each population from the flowering time of each individual in an early or late selection line in that population to account for effects of inter-generational environmental variation (Falconer and Mackay 1996; Burgess et al. 2007). A significant region by selection line interaction would indicate that response to selection on early and/or late flowering varied among northern, central, and southern regions. We implemented post-hoc Tukey HSD tests to examine pairwise differences among regions in response to selection on early and late flowering. All analyses were performed using the lme4 (Bates et al. 2014), lmerTest (Kuznetsova et al. 2014), lsmeans (Lenth 2014), and multcomp (Hothorn et al. 2008) packages in R 3.1.0 (R Core Team 2014), with two-tailed tests and significance assessed at $\alpha = 0.05$.

Accounting for bias in population size

Because focal populations varied in starting population size in the parental generation and subsequent generations, estimates of response to selection may appear to be lower in northern populations due to an artifact of having smaller sample sizes than central and southern

populations (Table 4.2). To account for this potential artifact, we created 3 additional selection lines from a random subset of 40 (out of 96) individuals of the parental generation of the C1 population. We repeated the same selection procedure described above on this random subset of individuals, and then compared the estimated response to selection from the subset of individuals with that based on all individuals to assess the potential effects of sample size on estimates of response to selection. We performed this procedure only for the C1 population because the C1 population exhibited a far greater difference in sample size when compared to northern populations than the C2 population (Table 4.2). The goal of creating selection lines based on a subset of C1 individuals was to assess whether lower response in northern vs. central populations could be attributed to an artifact of sample size alone, rather than to a biologically meaningful process that could potentially create a stable northern range limit.

Results

In the parental generation prior to selection, flowering time decreased with latitude (H = 189.5, df = 5, $P < 0.001$), with the northernmost population flowering on average 16 days earlier than the southernmost population (Fig. 4.2). Phenotypic variance in flowering time prior to selection ranged from 19.51 to 51.76 (Table 4.2). After 1 or 2 generations of selection, plants flowered later than those from the parental generation as a result of inter-generational environmental variation (Fig. 4.2). The cumulative weighted S ranged from 12.30 to 17.64 days earlier for early flowering lines, and from 19.48 to 23.38 days later for late flowering lines when compared to base populations (Table 4.1). After two generations of selection, in early flowering lines, plants flowered up to ~7 days earlier than control lines, while in late flowering lines, plants flowered up to ~12 days later than control lines, but the magnitude of response varied among populations (Table 4.2; Fig. 4.3).

Within-population tests for significant response to selection

Selection line was a significant predictor of flowering time in all populations except for N2 (Table 4.2; Fig. 4.3). In the N1 population, individuals from the early selection line had a significantly earlier mean flowering time than the control and late flowering lines, but the control and late flowering lines did not significantly differ in flowering time (Fig. 4.3). In the C1 population, individuals from the early flowering line flowered significantly earlier than those from the late flowering line, but flowering time in early and late flowering lines did not differ significantly from the control line (Fig. 4.3). In the C2 population, individuals from the late flowering line flowered significantly later than those from control and early lines, but there were not significant differences in flowering time between the early flowering and control lines (Fig. 4.3). In both the S1 and S2 populations, individuals from early flowering lines flowered significantly earlier than those from control and late flowering lines, and individuals from late flowering lines flowered significantly later than those from control lines after two generations of selection (Fig. 4.3).

Analyses comparing responses to selection among central and marginal populations

In the analysis assessing whether response to selection on early and late flowering varied among populations at the northern range edge, range center, and southern range edge, selection line ($F_{1,103.714} = 128.238$, $P < 0.001$) and region-by-selection line ($F_{2,103.636} = 41.952$, $P < 0.001$) had significant effects on flowering time, whereas region did not affect flowering time independently ($F_{2,2.579} = 0.430$, $P = 0.69$; Fig. 4.4). The significant region-by-selection line interaction suggests that the magnitude of response to selection varied among the northern edge, center, and southern edge of the species' range. Failing to support the prediction that northern populations should have lower responses to selection than central ones, there were no significant

differences in response to selection on early or late flowering time among northern and central populations (P = 0.996 and P = 0.856, respectively; Fig. 4.4). However, although not statistically significantly different, responses to selection on early and late flowering at the range center were slightly greater than those at the northern range edge based on least-square mean estimates (Fig. 4.4). Contrary to the prediction that southern populations should have lower responses to selection than central ones, populations at the southern edge of the range had a marginally significantly greater response to selection on early flowering and a significantly greater response to selection on late flowering than central and northern populations (early flowering: P = 0.087 and 0.058, respectively; late flowering: P = 0.003 and P < 0.001, respectively; Fig. 4.4). Based on least-square mean estimates, the magnitude of response to selection on early flowering in southern populations was ~5 days greater than that of central and northern populations, and the response to selection on late flowering in southern populations was ~7 days greater than that of central populations and ~9 days greater than that of northern populations.

Accounting for bias in population size

Similar to the full C1 population, there was a marginally significant effect of selection line on flowering time ($F_{2,11.998}$ = 3.679, P = 0.057) in the population consisting of selection lines built from 40 randomly sampled individuals of the C1 population. Raw mean flowering time in this subset of individuals was similar to that of the full C1 population in the parental generation (Fig. 4.5a vs. Fig. 4.2c, respectively). Nearly mirroring results from the full C1 population, individuals from the early flowering line flowered significantly earlier than plants from the late flowering line and marginally significantly earlier than plants from the control line (P = 0.051), and there were no differences in flowering time between late flowering and control lines (Fig. 4.5b). The magnitude of response to selection on early flowering was greater in the population

created from a random subset of C1 individuals, whereas the magnitude of response to selection on late flowering was lower than in the full C1 population (Fig. 4.5b vs. Fig. 4.3c, respectively), suggesting that smaller sample size alone did not predictably result in a lower magnitude of response to selection.

Discussion

Stable geographic limits may arise because populations at species' range margins lack sufficient genetic variation to respond to natural selection. We tested this hypothesis by quantifying response to selection on flowering time in two northern edge, two central, and two southern edge populations of *M. cardinalis*. We documented strong variation in response to selection across the species' geographic range. Contrary to prediction, populations from the southern range limit exhibited a significantly greater response to selection than populations at the center or northern limit of the species' range. After just two generations of artificial selection, the magnitude of response to selection on flowering time in southern populations was 3 times greater than that of central populations and 4-7 times greater than that of northern populations. Although not statistically significant, northern populations exhibited lower responses than central ones, consistent with the notion that reduced additive genetic variance constrains adaptation at range margins. Thus, we did not document consistent evidence for the hypothesis that marginal populations have less adaptive potential than populations at the center of species' range. While response to selection varied among populations across the range in our study, high- and low-latitude edges exhibited drastically different magnitudes of response, and as such, the patterns we observed cannot be clearly explained by range position alone. Below, we interpret these results in light of previous tests of this hypothesis in other taxa, potential biases that could drive the observed patterns, other studies testing range-limiting hypotheses in *M. cardinalis*, and

alternative mechanisms that may drive variation in response to selection on flowering time across geography. We conclude by discussing the implications of our findings in the context of understanding how populations across a species' range may respond to changing climate.

Previous tests of the hypothesis that edge populations exhibit lower genetic variation in ecologically important traits than central ones are equivocal. Some studies have failed to find support for this hypothesis (Jenkins and Hoffmann 2000; Kelly et al. 2012; Gould et al. 2014). For example, a study comparing quantitative genetic variation among populations across the geographic range of a *Clarkia* species did not document differences in heritability for flowering time or for five additional traits among populations, failing to support the hypothesis of reduced additive genetic variance in edge populations (Gould et al. 2014). On the other hand, other studies have found support for the hypothesis that edge populations have lower additive genetic variation than central ones (Blows and Hoffmann 1993; Etterson 2004; Pujol and Pannell 2008; Volis et al. 2014). For example, additive genetic variance in eastern and western edge populations of *Mercurialis annua* was much lower than that of populations from the historical core of the species' range (Pujol and Pannell 2008), providing strong support for the notion that adaptation in edge populations is constrained by insufficient ability to respond to selection.

In the present study, we documented a trend of response to selection on flowering time increasing from north to south. Variation in response could have been driven by variation in the strength of selection, but there is no evidence that selection differential and response to selection are positively related across populations (Table 4.2). Strikingly, the N2 population exhibited the greatest selection differentials, yet the lowest responses to selection (Table 4.2). Another potential cause of differences in responses to selection across populations is sample size of each parental generation and sample size within each selection line in subsequent generations. To test

for this potential sampling artifact, we performed the same routine of selection using a random sample of 40 individuals from the C1 population and found qualitatively similar responses as in the full C1 population. Thus, differences in response to selection in our study do not seem to be driven solely by sample size in the parental generations and subsequent selection lines.

Although variation in sample size did not drive variation in the magnitude of response to selection among populations, population size and dynamics in the natural populations from which seed was sampled could explain some variation in estimates of response to selection among populations. In general, the size of our study populations increased from north to south (Sheth & Angert, unpubl. data), which is why sample sizes in the parental generation increased from north to south (Table 4.2), and could explain the greater responses to selection in southern populations. When sampling seed for this study, to achieve adequate sample sizes for the artificial selection experiments, we were constrained to collect from localities with a large number of individuals, and thus likely did not include the smallest populations at the northern and southern edges, which should have had the lowest responses to selection. Although they were not included in our study due to the logistical reasons described above, we observed small populations with fewer than 40 adults at both the northern and southern edges of the range (Sheth & Angert, unpubl. data). The influence of the sizes of natural populations, however, is a biological effect rather than an artifactual one, because one of the main mechanisms by which edge populations should exhibit lower additive genetic variance and hence lower responses to selection is by having smaller population sizes than populations at the range center (Hoffman and Blows 1994).

The observation that the southern edge populations were the largest populations included in our study deviates from the expectation that geographic range limits are in equilibrium with

ecological niche limits and suggests that these southern populations, although geographically peripheral, may not occupy marginal niche space. Based on range-wide demographic surveys of *M. cardinalis*, which only include the N1, C2, and S1 populations from the present study, response to selection increases with asymptotic population growth rates (Sheth and Angert; unpubl. data). In fact, the S1 population had the highest population growth rate of any site included in the entire demographic survey of ~30 sites across the latitudinal range, with population size projected to increase over time (lambda significantly > 1), while the N1 population was projected to decrease over time (lambda significantly < 1; Sheth, and Angert; unpubl. data). Intermediate between the N1 and S1 populations, the C2 population exhibited stable population growth (lambda not significantly different than 1; Sheth, and Angert; unpubl. data). Further, according to climatic suitability estimated from climatic niche models, the northern populations had the lowest suitability scores, consistent with the notion that they occur at a niche limit (Bayly & Angert, unpubl. data). In contrast, the S1 population with the highest response to selection had the highest suitability score of our 6 study populations. The two central populations had intermediate climatic suitability scores that were only slightly lower than that of the S1 population, and the S2 population had only a slightly higher suitability score than the northern populations (Bayly & Angert, unpubl. data), inconsistent with estimates of response to selection. In sum, there is evidence beyond our study suggesting that the northern range edge populations in our study are demographic sinks occurring in climatically marginal habitat, whereas the southern range edge populations in our study constitute demographic sources with variable climatic suitability.

If genetic drift associated with small population size leads to low additive genetic variance in edge populations, then populations with low additive genetic variance should also

exhibit low neutral genetic variation (Hoffman and Blows 1994). A study of neutral genetic structure based on 49 populations across the geographic range of *M. cardinalis*, including all of our study populations except for the C2 population, reveals a pattern of within-population neutral genetic variation decreasing towards the northern and southern range edges and among-population genetic differentiation increasing from the latitudinal range center towards the northern range edge (Paul et al., in prep). According to this work, the northern populations in our study have lower neutral genetic variation than the central and southern study populations, further supporting the idea that northern populations exhibited low responses to selection at least partially as a result of genetic drift associated with small population size (Paul et al., in prep). Paul et al. (in prep) detect a significant genetic break between the S1 and S2 populations and the remaining populations in our study such that F_{st} is an order of magnitude lower (indicating potentially more gene flow) among northern and central populations than among southern and non-southern populations. This rules out the possibility that asymmetrical gene flow from the range center has increased genetic variation in the south and resulted in high responses to selection in the southern populations. Explicit tests of the swamping gene flow hypothesis that formally quantify asymmetrical gene flow among range positions of *M. cardinalis* would shed light on the interplay between gene flow and additive genetic variance across species' ranges.

Latitudinal variation in response to selection on flowering time may be explained by variation in the strength and form of natural selection across the species' range. For example, there is ample evidence of strong directional selection on flowering time at high latitudes (Munguía-Rosas et al. 2011), favoring early flowering perhaps to ensure that plants mature fruits before the growing season ends and potentially depleting genetic variation in flowering time. However, some have argued that there may be stabilizing selection on flowering time because

plants that flower early may be smaller, have fewer resources to invest towards fecundity, experience greater risk to frost damage (Inouye 2008) and/or exhibit pollinator limitation (Thomson 2010), whereas plants that flower late may not have sufficient time to mature fruits before the growing season is over (Anderson et al. 2011). At low latitudes where the growing season is longer, it is possible that flowering time is under weaker selection, thereby maintaining additive genetic variance (Munguía-Rosas et al. 2011). A major assumption of our study is that flowering time is under selection at both the northern and southern range margins, and that evolution of flowering time would permit range expansion. However, for genetic variation in flowering time to constrain adaptation at range edges, flowering time must be associated with fitness in natural edge populations. Thus, quantifying the strength and form of selection on flowering time across the range of *M. cardinalis* would help to further explain the spatial variation in responses to selection observed here. Even though southern populations exhibited ample genetic variation in flowering time, they may lack necessary variation in multiple traits to effectively respond to natural selection (Walsh and Blows 2009). Trade-offs between flowering time and other traits may constrain adaptation, for example if selection favors high values of negatively correlated traits (Etterson and Shaw 2001). If genetic correlations that are antagonistic to the direction of selection are present in marginal populations, then they may contribute to stable range limits (Blows and Hoffmann 2005). In fact, the magnitude of response to selection on single traits may be far larger than response to selection after accounting for correlations among traits (Etterson and Shaw 2001). Hence, to better understand the potential for multivariate constraints at range edges, it would also be beneficial to examine whether other traits exhibit correlated responses in selection on early or late flowering that are antagonistic to the direction of natural selection.

Quantifying adaptive potential in traits that are likely targets of selection during climatic changes has several important implications for understanding range-wide populations' responses to climatic change (Shaw & Etterson 2012). Before industrialization and the onset of contemporary anthropogenically caused climate change, there may have been stabilizing selection on flowering time, but contemporary studies suggest that climate change is imposing increasingly strong directional selection for early flowering (as growing seasons begin earlier), such that populations with insufficient genetic variation to respond to selection on flowering may lie far from their fitness optima and thus be vulnerable to extinction (Miller-Rushing and Primack 2008; Anderson et al. 2012). Moreover, such effects may be compounded if strong directional selection depletes standing additive genetic variance in flowering time (Anderson et al. 2012), and ultimately population responses will depend on whether populations' rates of adaptive evolution exceed rates of climate change. Our study suggests that low-latitude, trailing edge populations have ample genetic variation to respond to natural selection on flowering time in just a few generations, whereas high-latitude populations at the leading edge and latitudinally central populations may be unable to adapt quickly enough to keep up with climate change. Thus, while the southern populations in our study may be able to adapt to changing conditions in situ, the individuals from the northern and central populations will likely have to migrate northward to track climatically favorable conditions. Given the high adaptive potential observed in the southern edge populations in our study, we argue that not all geographically peripheral populations are particularly vulnerable to climate changes. Instead, in our study, northern populations merit conservation attention due to their lack of adaptive potential, whereas southern populations warrant consideration because they represent a source of adaptive potential (Vucetich and Waite 2003). Studies of species' range shifts associated with climate change often

link species' occurrences to current climate data to model the climatic niche, which is then projected onto geography based on estimates of future climate (Pearman et al. 2008). Yet, these models do not typically account for the potential for evolutionary change or for variation in adaptive potential among populations of a species (Pearman et al. 2008). Further, because global change constitutes both anthropogenic alterations to climate and habitat fragmentation associated with anthropogenic activities, mechanistic studies of constraints to adaptation may become more applicable than purely correlative studies of predicted range shifts. Lastly, while community- and ecosystem-level responses to climate change are of tremendous interest, communities are made up of populations of individual species. A thorough understanding of higher-order responses therefore requires a consideration of the individualistic responses of these populations, which, in turn, may depend on their evolutionary potential. These results provide critical insights about how spatial variation in adaptive potential affects population persistence in the face of climate change.

Table 4.1 Information for localities of *M. cardinalis* from northern range edge, range center, and southern range edge. Latitude and longitude are in units of decimal degrees; elevation is in units of meters; $\overline{T_{sm}}$: mean summer temperature (June – August) in ^{o}C; PCT_{sm}: summer precipictation (June – August) in mm. Temperature and precipitation variables were extracted as variables averaged across 1981-2009 from the web version of climateWNA (Wang et al. 2012). The N1, N2, and S2 sites occur on lands managed by the Bureau of Land Management, the C1 and C2 sites occur in Yosemite National Park, and the S1 site occurs in Cuyamaca Rancho State Park.

Region	Population	Latitude	Longitude	Elevation	$\overline{T_{sm}}$	PCT_{sm}	Drainage
north	N1	43.37876	-122.95207	295	19.2	88	Rock Creek
north	N2	42.53529	-123.73016	914	17.4	118	North Fork Silver Creek
center	C1	37.70377	-119.75363	1316	20.9	24	Crane Creek
center	C2	37.54576	-119.64152	1228	21.3	38	South Fork Merced River
south	S1	32.92788	-116.56019	1252	21.1	46	Cold Stream
south	S2	32.60831	-116.70098	252	25.2	21	Cottonwood Creek

Table 4.2 Summary of linear mixed-effects analyses of variance of flowering time (number of days from germination to first flower) in each population, with selection line (early flowering, control, or late flowering) as a fixed effect and family as a random effect. Number of individuals (N_{ind}) and families (N_{fam}) planted for each selection line the final generation (2014); cumulative weighted selection differential (*S*); cumulative *R* (estimated as least-square mean difference between early or late flowering line and control line after 2 generations of artificial selection); realized heritability (h^2) and additive genetic variance (V_A) after two generations of artificial selection; phenotypic variance in 2012 parental population (V_P) in flowering time; denominator degrees of freedom (df_{den}) based on Satterthwaite's approximation; and *F* test-statistic used to assess significance of fixed effects. Numerator degrees of freedom = 2 in each population.

			S		*R*				
Population	N_{ind}	N_{fam}	early	late	early	late	V_P	df_{den}	*F*
N1	40	5	-12.30	21.77	-4.30	-0.15	25.86	12.209	11.366**
N2	48	6	-17.64	23.38	1.25	2.86	31.02	15.039	1.755
C1	96	12	-15.37	22.64	-2.78	1.66	19.51	32.689	6.323**
C2	64	8	-12.48	21.55	-1.53	4.99	26.88	21.136	7.545**
S1	104	13	-13.72	19.48	-7.21	11.80	31.13	36.032	71.302***
S2	104	13	-15.69	20.31	-6.40	8.46	51.76	36.097	22.612***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

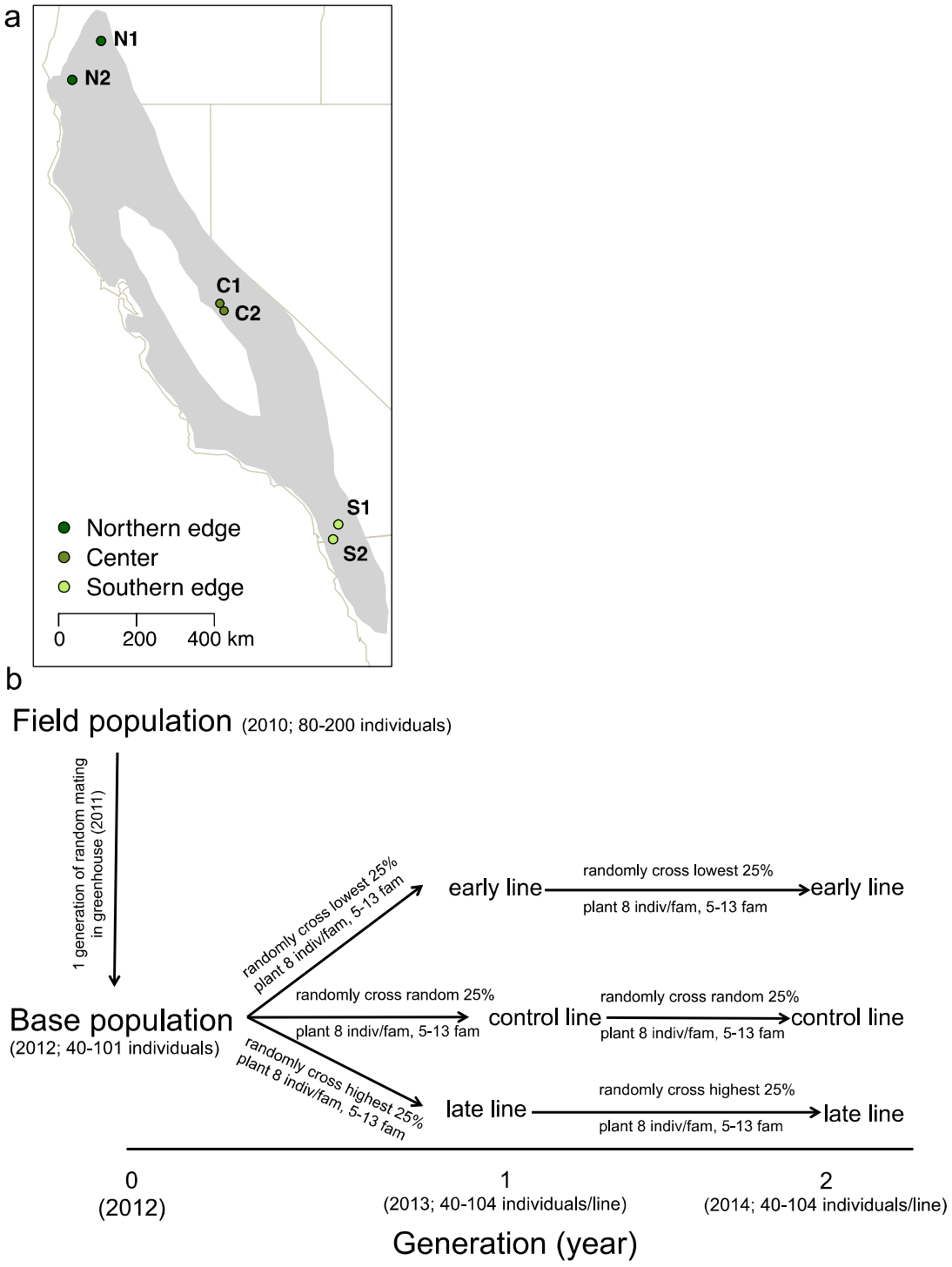

Figure 4.1 (a) *M. cardinalis* range map (shown in gray; modified from Hiesey et al. 1971) with populations sampled at the northern range edge (N1 & N2), range center (C1 & C2), and southern range edge (S1 & S2). (b) Diagram of experimental design of artificial selection program for each population (N1, N2, C1, C2, S1, and S2). Within each population, seed collected from individuals in the field were first randomly crossed for one generation in the greenhouse to reduce maternal effects. The resulting seed was planted to produce the base

parental population within each of the 6 study populations. Subsequently, two generations of selection on early flowering and late flowering were performed, and an unselected control line was also maintained during this time. In each generation of selection, 8 individuals (abbreviated as “indiv”) per full-sibling family (abbreviated as “fam”) were planted to maintain similar sample sizes across generations. See Methods for further details.

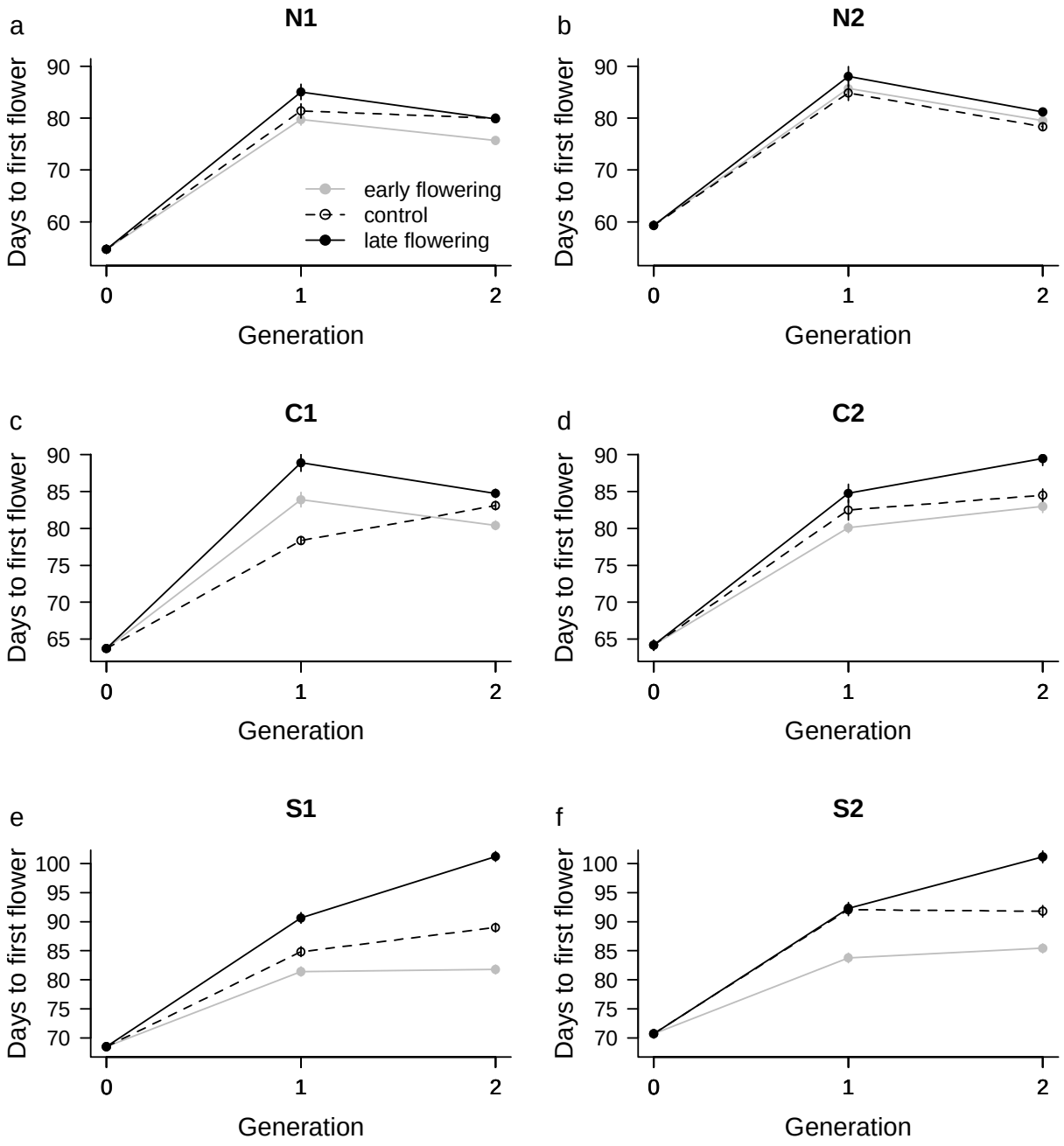

Figure 4.2 Change in raw mean flowering time ± 1 SE from one generation to the next for each population of *M. cardinalis*.

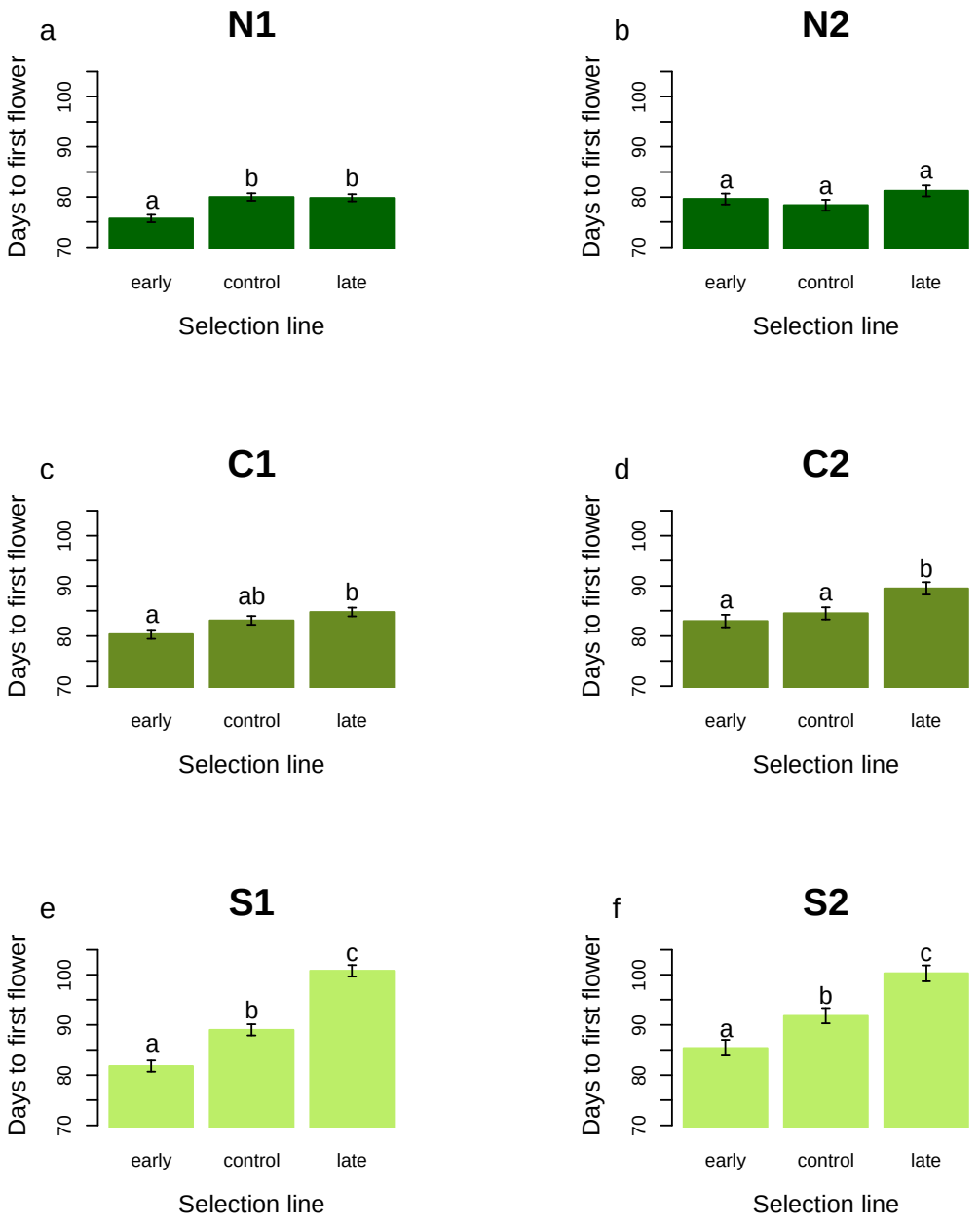

Figure 4.3 Least-square mean estimates of flowering time in each selection line in each population of *M. cardinalis* ± 1 SE after two generations of selection. Different letters represent significantly different means in each population ($\alpha = 0.05$).

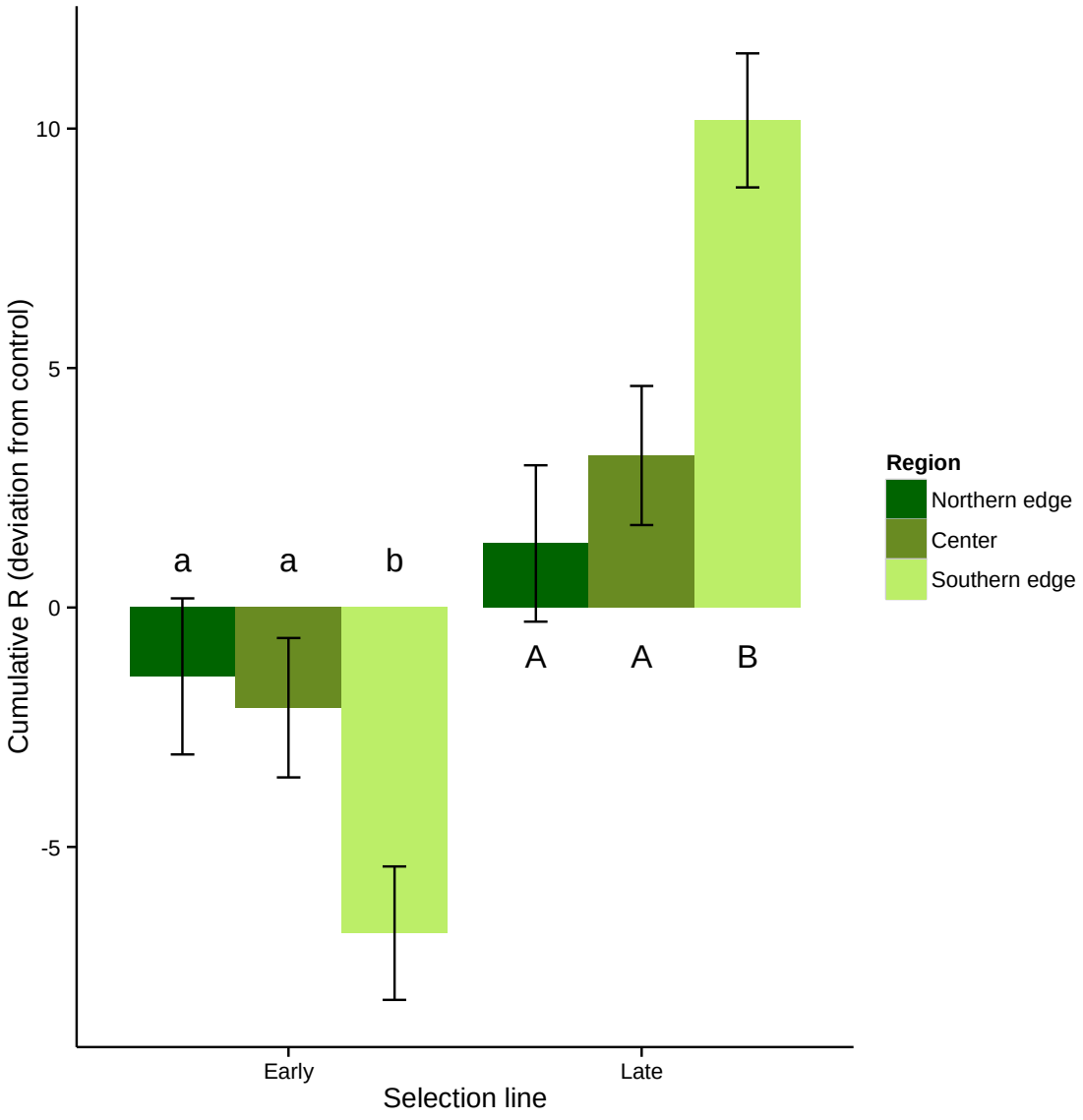

Figure 4.4 Cumulative response to selection ± 1 SE on early and late flowering, expressed as deviations in number of days from the mean flowering time of control lines, for each region based on least-square means. For early flowering lines, different lowercase letters represent marginally significantly different means in each region (for southern edge vs. center comparison, $P = 0.059$, and for southern vs. northern edge comparison, $P = 0.087$), and for late flowering lines, different uppercase letters represent significantly different means in each region (for southern edge vs. center comparison, $P < 0.001$, and for southern vs. northern edge comparison, $P = 0.003$).

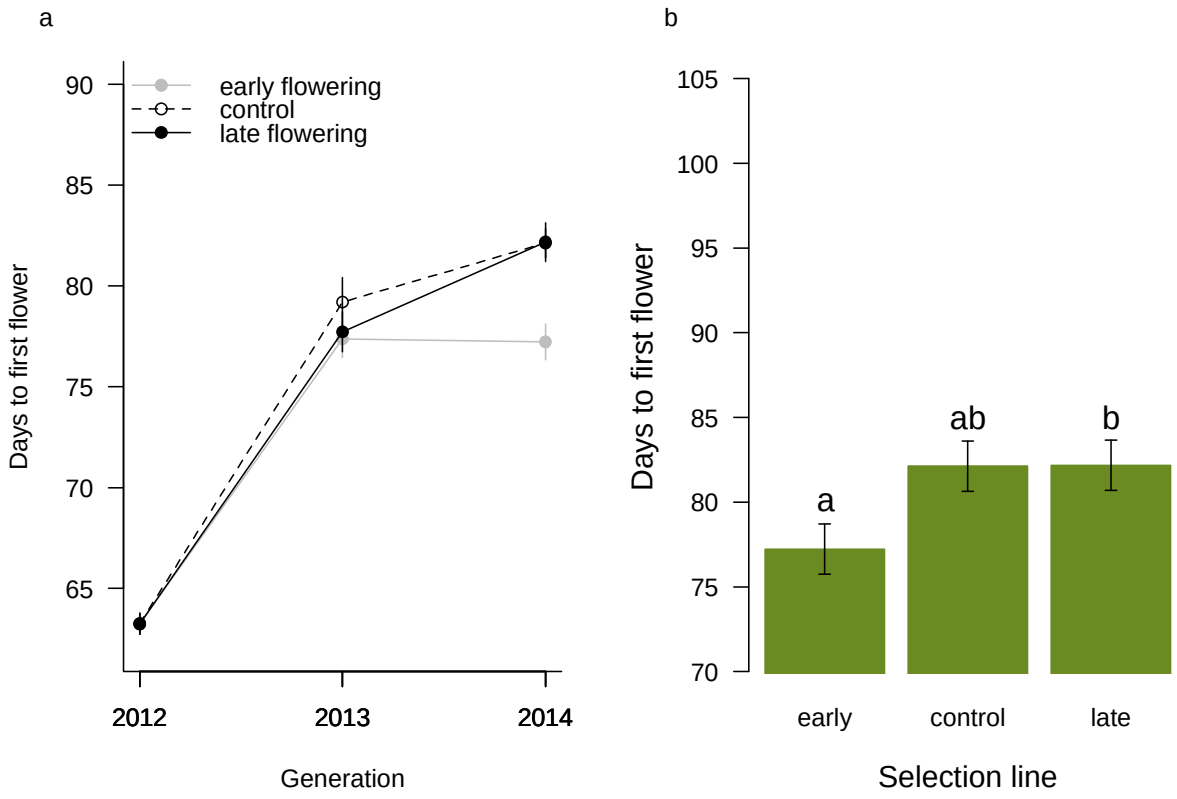

Figure 4.5 (a) Change in raw mean flowering time ± SE from one generation to the next for selection lines created from 40 randomly sampled individuals from the C1 population of *M. cardinalis*. (b) Least-square mean estimates of flowering time in each selection line created from 40 randomly sampled individuals from the C1 population ± SE. Different letters represent significantly different means ($\alpha = 0.05$).

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5. CONCLUSIONS AND SYNTHESIS

The overall goal of my dissertation was to study the mechanisms underlying the sizes and limits of the geographic distributions of western North American *Mimulus* species. In the first study of my dissertation, I assessed the relative importance of climatic niche properties and connectivity of climatically suitable habitat in explaining variation in the amount and occupancy of climatically suitable habitat, respectively, and in turn, variation in geographical range size. I documented strong support for the hypothesized effects of climatic niche breadth, but not niche position and connectivity of climatically suitable habitat. To my knowledge, this is the first study to show that climatic niche breadth, via its effects on the amount of climatically suitable habitat, is a strong predictor of geographical range size, thereby improving our understanding of the mechanisms driving species rarity. Specifically, my results suggest that niche breadth is the best predictor of range size in western North American monkeyflowers, providing strong evidence that species with narrow climatic niches have a limited ability to achieve large ranges. These results set the stage for detailed experimental investigations of relationships among range size and niche breadth in a subset of western North American *Mimulus* species.

Thus, in the second study of my dissertation, I used five pairs of western North American monkeyflowers to experimentally test the niche breadth hypothesis by quantifying performance across eight temperature regimes. I also examined the role of climatic variability, specialist-generalist tradeoffs, plasticity, and quantitative genetic variation in shaping patterns of thermal tolerance in these species pairs. This study complements my conclusions from the first study by showing that in four out of five of the focal species pairs, the widespread species had a broader thermal performance curve than the geographically restricted species, providing additional

support for the niche breadth hypothesis. With the exception of *M. cardinalis* and *M. parishii*, estimates of thermal performance breadth for widespread versus restricted species derived from one population per species (in the second study) are consistent with estimates of multivariate climatic niche breadth from correlative modeling (in the first study). As predicted, species with broader thermal tolerances also had more genetic variation in thermal reaction norms and experienced greater thermal variation across their geographic ranges than species with narrow thermal tolerances. In three species pairs, species with greater phenotypic plasticity also had broader thermal tolerances, but only one species pair supported the expectation of specialist-generalist tradeoffs.

Although in the second study genetic variation for thermal performance within a single population per species affected environmental tolerance, genetic variation in ecologically important traits may vary across species' ranges. Theoretical and empirical work suggests that populations at the edges of species' ranges may lack genetic variation in one or more ecologically important traits (Antonovics 1976, Pujol and Pannell 2008). Thus, for the third study of my dissertation, within one widely distributed *Mimulus* species, *M. cardinalis*, I estimated response to artificial selection on flowering time across the species' range to further understand how genetic variation may promote or hinder niche evolution and range expansion. I documented spatial variation in response to selection on flowering time, but contrary to prediction, southern populations exhibited significantly greater responses to selection on flowering time than central or northern populations. Yet, there was a trend (but not statistically significant) of northern populations having lower responses than populations from the range center. Based on these results, while the northern populations may be approaching a niche limit, the southern populations, although geographically peripheral, probably do not occur in marginal

niche space. In the second study of my dissertation, I found that a southern population of *M. cardinalis* (near Palm Springs, California) had less quantitative genetic variation and a narrower thermal tolerance than a population of a closely related but more narrowly distributed species, *M. parishii*, from the same locality. Given the spatial variation in adaptive potential observed in the third study of my dissertation, environmental tolerance may also vary across the range of *M. cardinalis*, explaining why *M. cardinalis*, despite being more widespread, had a narrower environmental tolerance than *M. parishii*. Ultimately, the results from the second and third studies provide the foundation with which to examine how quantitative genetic variation interacts with natural selection, and in turn, affects fitness and population dynamics.

Niche breadth (analogous to "habitat specificity" in some literature) and geographic range size constitute two axes of rarity (Rabinowitz 1981) that may impact extinction risk. In this dissertation research, a strong relationship between niche breadth and geographic range size indicates that species with narrow niches and small geographic ranges may be doubly at risk of extinction. Moreover, species with narrow environmental tolerance may be particularly vulnerable to changing climatic conditions due to lack of plasticity and insufficient genetic variation to respond to novel selection pressures. Finally, genetic variation in ecologically important traits played an important role in constraining the evolution of broad environmental tolerance in *Mimulus* species, but adaptive potential varied across the geographic range of *M. cardinalis*, highlighting the importance of quantifying environmental tolerance for multiple populations across species' ranges. The results of this dissertation research provide critical insights into how variation in adaptive potential among populations or species may influence responses to environmental change.

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Pujol, B. and J. R. Pannell. 2008. Reduced responses to selection after species range expansion. Science **321**:96.

Rabinowitz, D. 1981. Seven forms of rarity. Pages 205-217 *in* H. Synge, editor. The Biological Aspects of Rare Plant Conservation. Wiley, New York.

APPENDIX 1.1

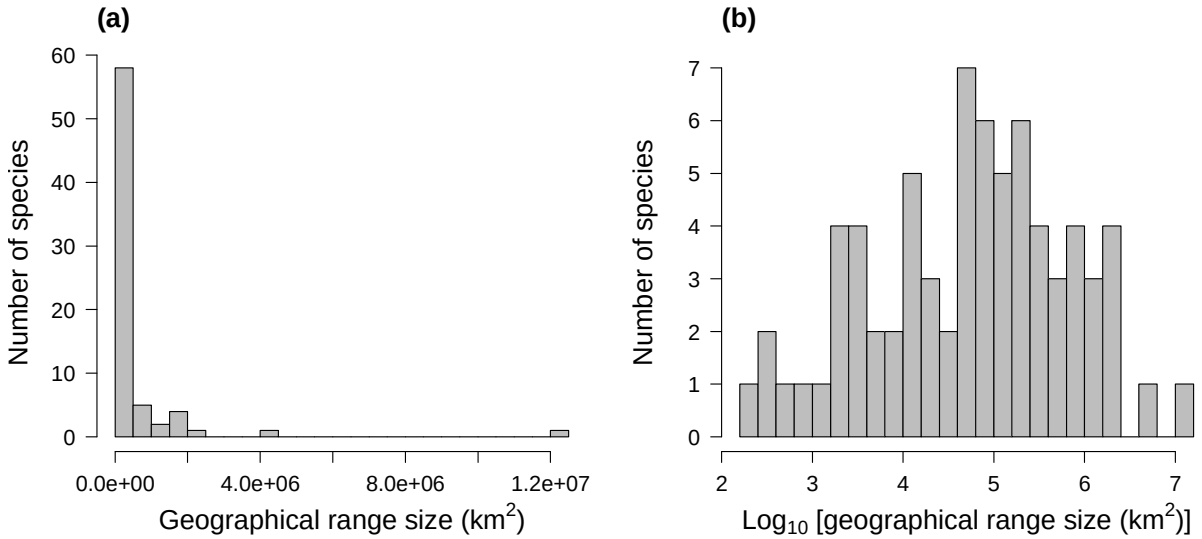

Figure S1.1 (a) Untransformed and (b) log-transformed frequency distribution of geographical range size (measured as extent of occurrence) for western North American *Mimulus* species.

Figure S1.2 Western North American *Mimulus* occurrence data used to estimate geographical range size, niche properties, connectivity and amount and occupancy of climatically suitable habitat (shown with Albers equal area conic projection of North America).

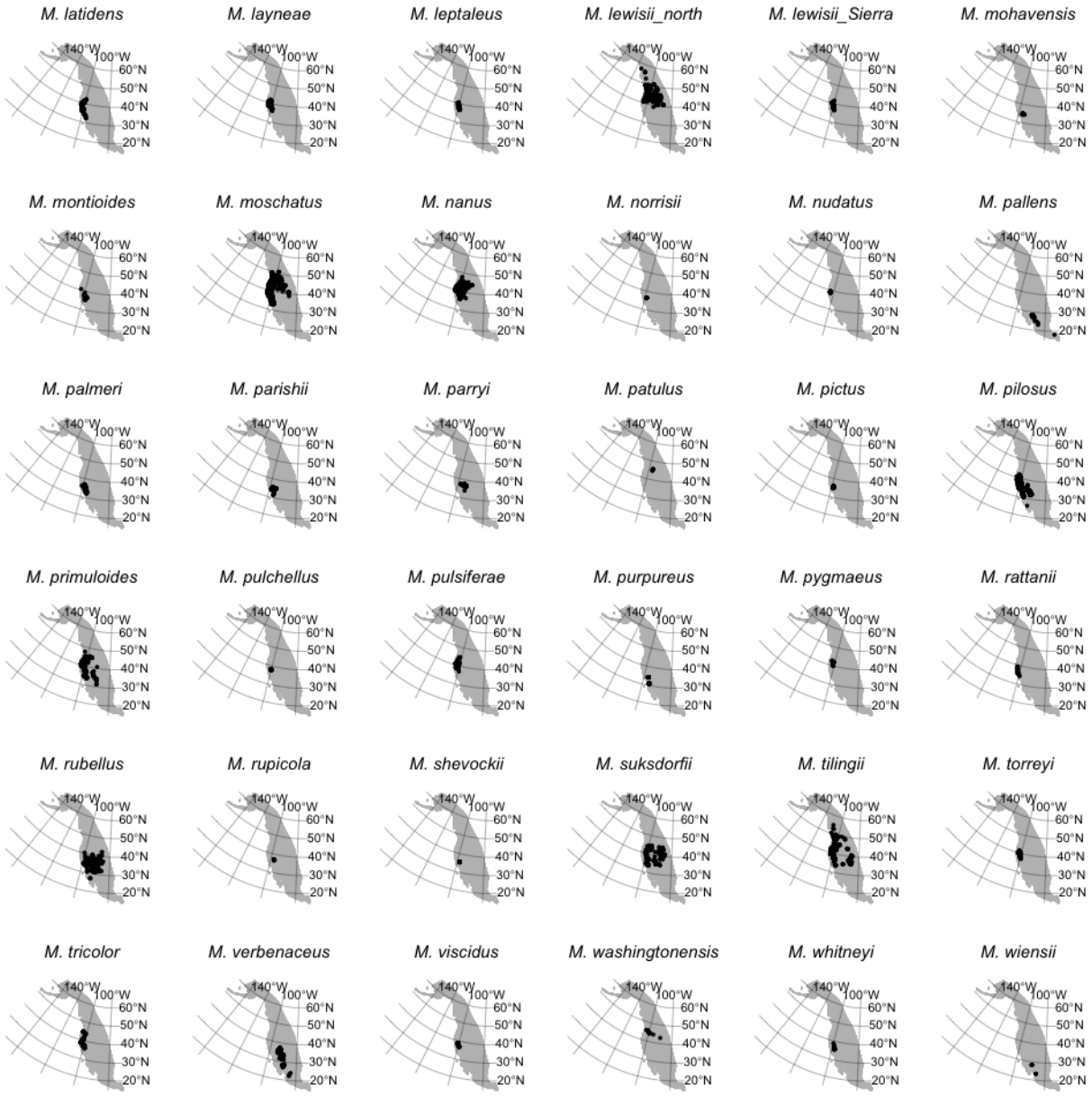

Figure S1.2 (continued)

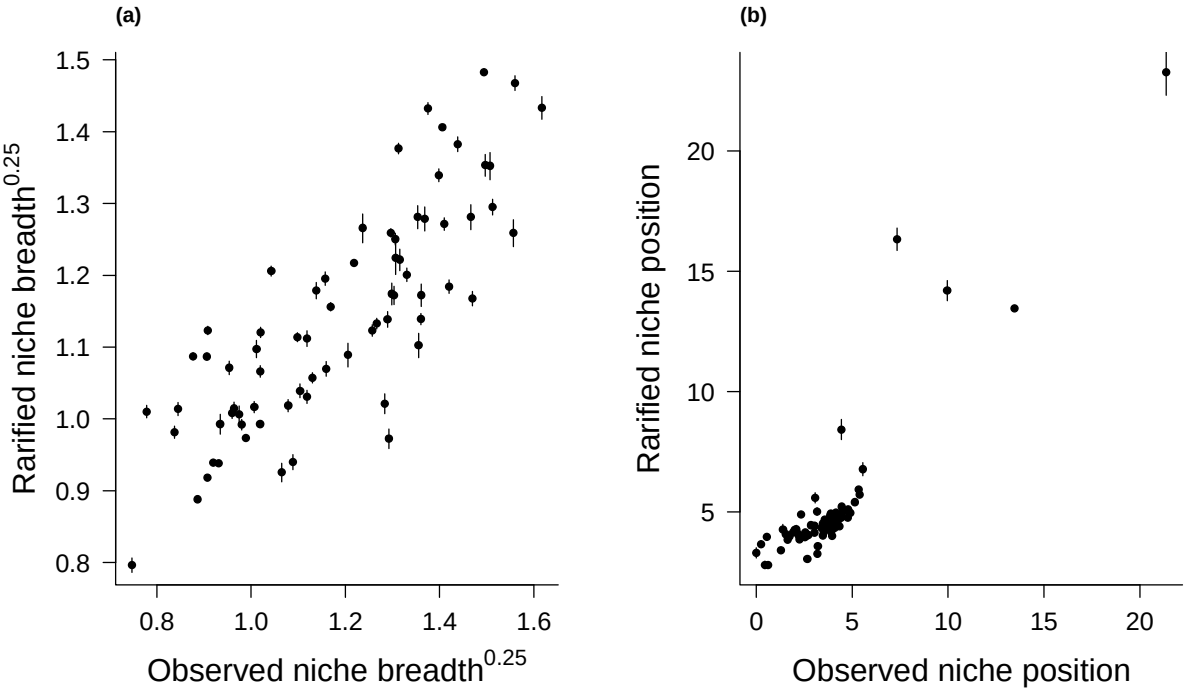

Figure S1.3 Relationship between observed niche breadth and position derived from MAXENT models built from all occurrence records and rarefied niche breadth and position (± 1 standard error) derived from MAXENT models built from random subsamples of occurrence data of western North American *Mimulus* (see Materials and Methods for details).

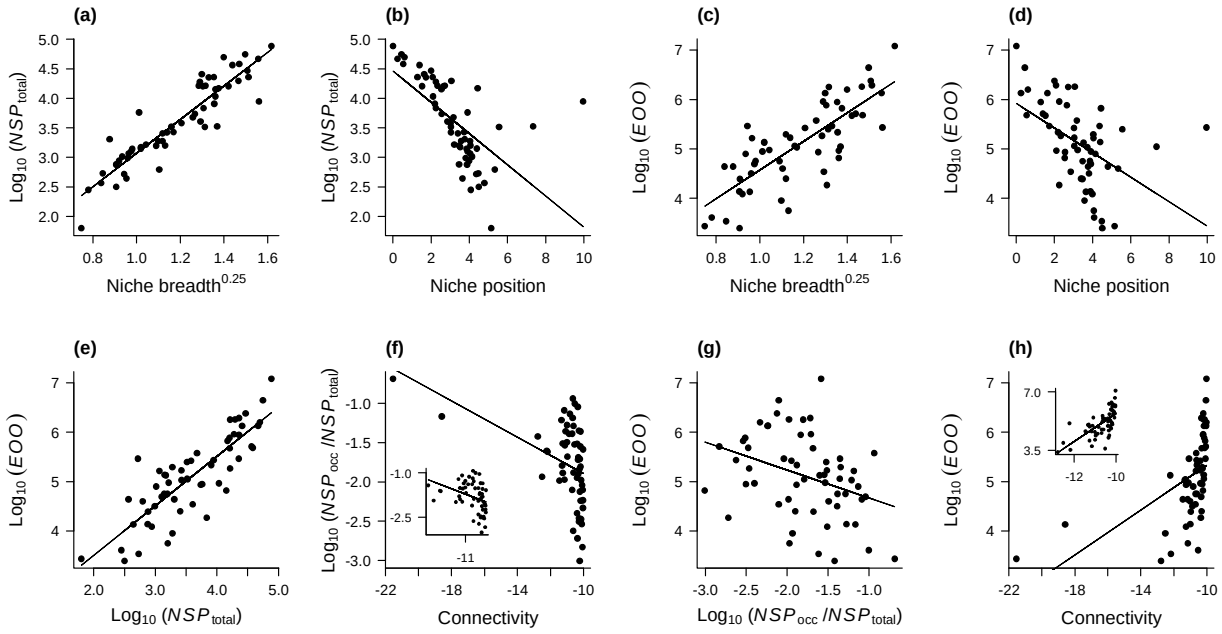

Figure S1.4 Simple linear regressions of $\log_{10}$ (NSP_{total}) and $\log_{10}$ (EOO) on (a, c) niche breadth and (b, d) niche position, respectively, and of (e) $\log_{10}$ (EOO) regressed on $\log_{10}$ (NSP_{total}), excluding western North American *Mimulus* species occupying fewer than 10 grid cells. Inset panels show relationships with outliers removed. Simple linear regressions of (f) $\log_{10}$ (NSP_{occ}/NSP_{total}) on connectivity of climatically suitable habitat, and of (g, h) $\log_{10}$ (EOO) on $\log_{10}$ (NSP_{occ}/NSP_{total}) and connectivity of climatically suitable habitat, respectively, excluding species occupying fewer than 10 grid cells. Inset panels show relationships with outliers removed. See Table S2.3 for definitions of abbreviations.

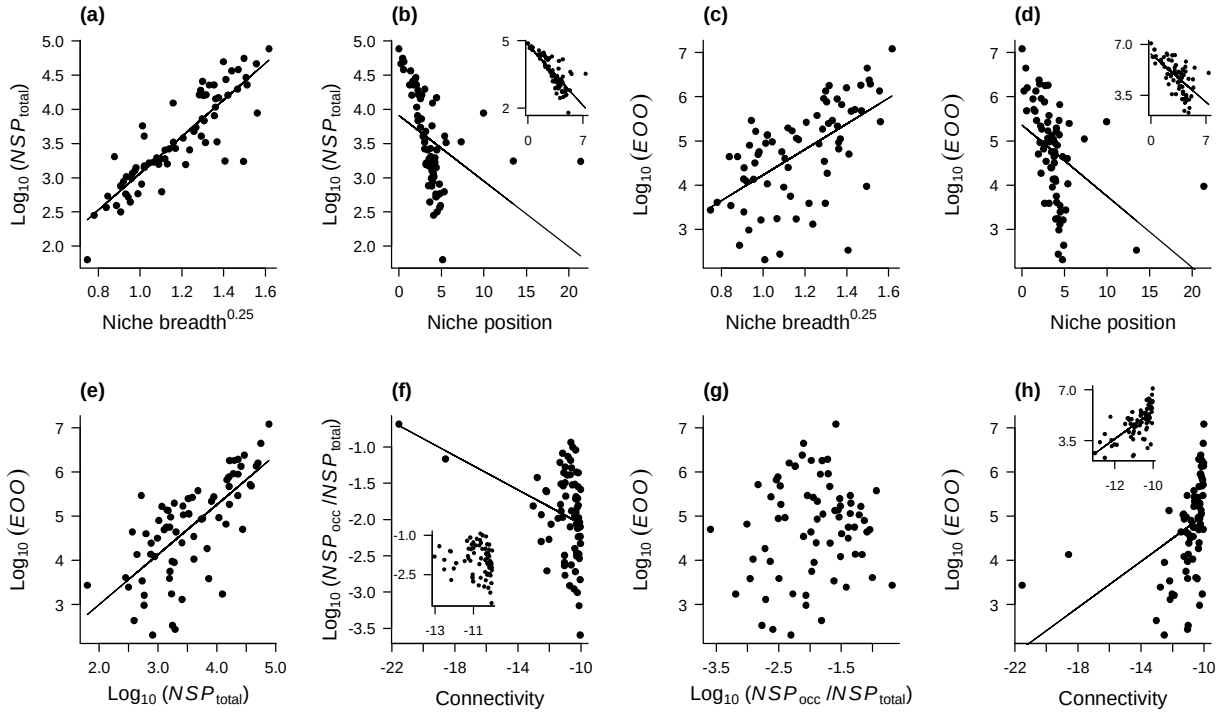

Figure S1.5 Simple linear regressions of $\log_{10}$ (NSP_{total}) and $\log_{10}$ (EOO) on (a, c) niche breadth and (b, d) niche position, respectively, and of (e) $\log_{10}$ (EOO) regressed on $\log_{10}$ (NSP_{total}), including the full set of 72 species of western North American *Mimulus*. Inset panels show relationships with outliers removed. Simple linear regressions of $\log_{10}$ (NSP_{occ}/NSP_{total}) on connectivity of (f) climatically suitable habitat, and of $\log_{10}$ (EOO) on (g, h) $\log_{10}$ (NSP_{occ}/NSP_{total}) and connectivity of climatically suitable habitat, respectively, including the full set of 72 species. See Table S2.3 for definitions of abbreviations and Table S2.5 for summaries of regression models.

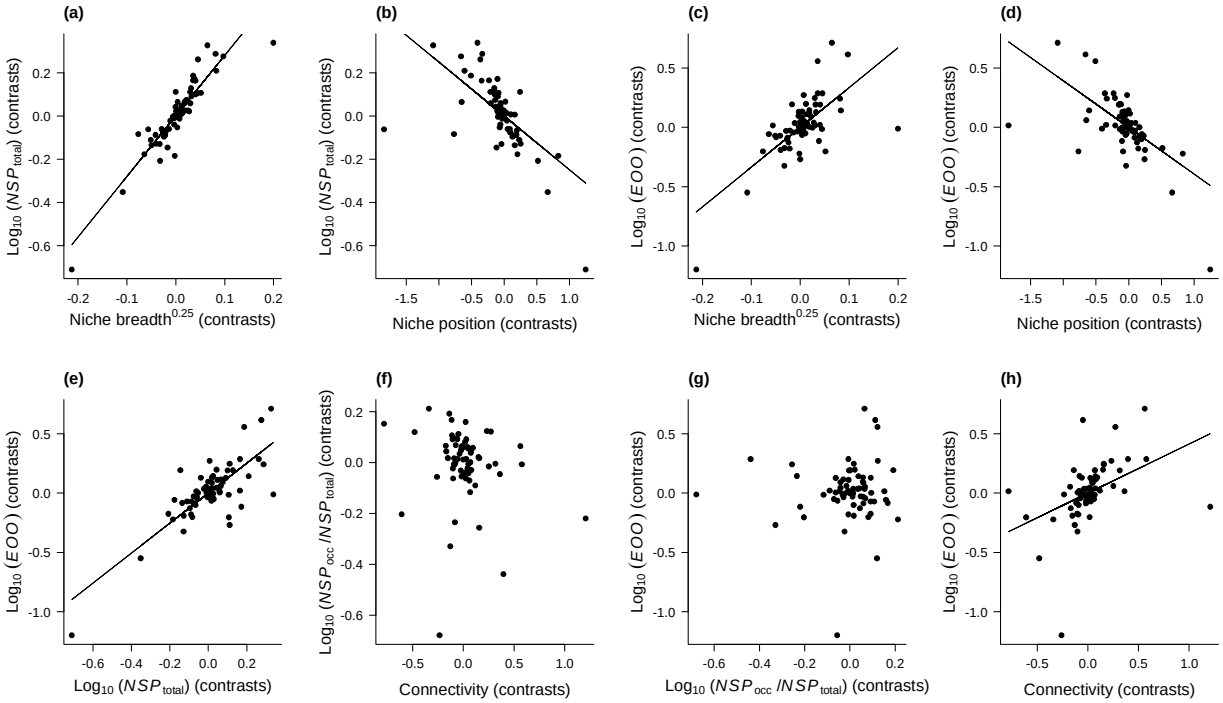

Figure S1.6 Simple linear regressions of $\log_{10}$ (NSP_{total}) and $\log_{10}$ (EOO) on (a, c) niche breadth and (b, d) niche position, respectively, and of (e) $\log_{10}$ (EOO) regressed on NSP_{total}, based on phylogenetically independent contrasts regressed through the origin (contrasts shown are among western North American *Mimulus* species). Simple linear regressions of $\log_{10}$ (NSP_{occ}/NSP_{total}) on connectivity of (f) climatically suitable habitat, and of (g, h) $\log_{10}$ (EOO) on $\log_{10}$ (NSP_{occ}/NSP_{total}) and connectivity of climatically suitable habitat, respectively, based on phylogenetically independent contrasts regressed through the origin. See Table S2.3 for definitions of abbreviations and Table S2.6 for summaries of regression models.

APPENDIX 1.2

Detailed methods

Taxonomic issues

Although western North American *Mimulus* is currently undergoing a taxonomic revision (Barker *et al.*, 2012), the proposed changes are primarily to nomenclature and retain the major patterns of the phylogenetic hypothesis used here. Notable exceptions that may affect our analyses include splitting of widespread species (e.g. M. *guttatus* and *M. moschatus*) into multiple species. The proposed splits are based primarily on morphological rather than molecular data, and they have not been widely adopted. Furthermore, it is not possible to map these very recent changes in taxonomy onto the georeferenced specimens used here. However, we conducted all the analyses with these potentially problematic species excluded and the results were qualitatively similar, suggesting that our results were not driven by a few widespread species with problematic taxonomy. This is probably because the new delineations retain a widespread species with a few, newly offshooting, narrowly distributed species whose limited number of distribution records do not substantially alter the estimation of parameters for the originally named widespread species. For species occurring in California, we adopted the taxonomic treatment used in Thompson (2011), and for species that do not occur in California we used accepted names from the International Plant Names Index (http://www.ipni.org; accessed in June 2010), except when there were published phylogenetic studies strongly suggesting an alternative treatment.

We split *M. lewisii* into two species (Sierra Nevadan and northern) based on recommendations in Beardsley *et al.* (2003). For *M. cardinalis*, we eliminated occurrences from

the south-western USA outside of California and central Mexico, because these populations have partial crossing barriers with *M. cardinalis* that occurs across the majority of the species' range from Oregon to northern Baja, California (Hiesey *et al.*, 1971; Beardsley *et al.*, 2003). *Mimulus dudleyi* was included as a separate taxon from *M. floribundus* because of evidence suggesting substantial morphological and genetic divergence (Beardsley *et al.*, 2004; Whittall *et al.*, 2006). Finally, the *M. palmeri* clade has recently undergone taxonomic changes (Fraga, 2012), thus we excluded specimens determined to species or subspecies as *diffusus* (listed in Thompson, 2011, as a synonym for *M. palmeri*) or *barbatus* (listed in Thompson, 2011, as a synonym for *M. montiodes*), which have been described as new species (Fraga, 2012).

Exploratory approach to structural equation modelling

Inspection of modification indices suggested that including paths connecting connectivity and amount of suitable habitat and niche breadth and occupancy of suitable habitat would greatly improve the model fit. Thus we added a path from amount of suitable habitat to connectivity to incorporate the possibility that species with more available suitable habitat have higher connectivity among pixels of suitable habitat than species with less available suitable habitat. Specifically, when the amount of suitable habitat is small, suitable habitat for a species can be distributed across the spatial extent of the study region in several ways, ranging from one or a few cohesive patches to several isolated patches. As the amount of suitable habitat increases, the number of possible distributions of suitable habitat across the study region decreases. This geometric constraint mechanism may cause an increase in connectivity as the amount of suitable habitat increases. Because we did not have a similar biologically plausible directional prediction for the relationship between niche breadth and occupancy of suitable habitat, we included a correlation between niche breadth and occupancy of suitable habitat in our modified model.

When we included a directional path from amount of suitable habitat to connectivity and a correlation between niche breadth and occupancy of suitable habitat, the observed co-variance matrix from our dataset significantly deviated from the modified model (Satorra–Bentler corrected $\chi^2 = 33.811$, d.f. = 7, $P < 0.001$). However, when including correlations between amount of suitable habitat and connectivity and between niche breadth and occupancy of suitable habitat, the observed covariance matrix from our dataset did not deviate significantly from this modified model (Fig. 2b).

Null model

We used a null model in which we shifted the geographical centroid of the occurrences of each species to a randomly chosen point within the study region. Subsequently, the occurrences were rotated about their new centroid by a randomly chosen angle. We repeated this process of shifting and rotating the occurrences of each species until we obtained 100 randomized distributions for which all occurrences fell onto land within the study region, using the SP 1.0-14 (Pebesma & Bivand, 2005) and RASTER 2.1-66 (Hijmans, 2013) packages in R. Thus, these randomized distributions preserved all aspects of the spatial structure of the occurrence data of each species, except their location within the study region. We built a MAXENT model for each of the 100 randomized distributions of the occurrence data of each species, and calculated niche breadth, niche position, connectivity and amount and occupancy of suitable habitat using the same procedures described for our dataset of observed occurrences. The spatial structure of the occurrence data of *M. guttatus* and *M. floribundus* prevented shifting and rotating occurrence data within the study region. Given the very limited number of ways the occurrence data for these species could be distributed in the study region, we assumed that estimates of niche properties, connectivity and amount and occupancy of suitable habitat derived from randomized

distributions of occurrence data closely matched observed estimates. Thus, for these two species, we assigned the same observed estimate of each variable to each of the 100 randomized datasets.

Phylogenetic signal and phylogenetically independent contrasts

We performed four commonly used tests for phylogenetic signal in each explanatory and response variable (Münkemüller *et al.*, 2012). First, we implemented tests of serial independence (Abouheif, 1999) with 1000 permutations in the ADEPHYLO 1.1-6 package (Jombart & Dray, 2008) in R. We chose this method because it does not require branch lengths, it does not assume an explicit model of evolution, and it can handle polytomies (Abouheif, 1999). In addition, we used Blomberg's K (Blomberg *et al.*, 2003) and Pagel's lambda (Pagel, 1999), which assume that traits evolve according to a Brownian motion model. We used the PHYTOOLS 0.3-72 package (Revell, 2012) to test the null hypothesis of no phylogenetic signal (indicated by lambda or K = 0), using a likelihood ratio test in the case of Pagel's lambda and a randomization test (with 1000 randomizations) in the case of Blomberg's K. The fourth method we used to test for phylogenetic signal was by comparing the fit of Brownian motion, Ornstein–Uhlenbeck and white noise models of evolution for each variable in our structural equation models using the GEIGER 1.99-3.1 package (Harmon *et al.*, 2008) in R. We used the sample size-corrected Akaike information criterion to assess model fit.

To conduct phylogenetically controlled analyses, we first arbitrarily resolved all polytomies in the tree and then obtained phylogenetically independent contrasts (Felsenstein, 1985) with the APE 3.0-11 (Paradis *et al.*, 2004) and GEIGER packages in R. We were unable to transform branch lengths to meet the assumption that the absolute values of standardized contrasts are unrelated to their standard deviations (Garland *et al.*, 1992), but phylogenetically independent contrasts are generally robust to violations of such assumptions (Díaz-Uriarte & Garland, 1996). In addition to

simple regressions testing the hypothesized causal relationships depicted in Fig. 2b, we also conducted correlation tests for other pairs of variables (Fig. 2b), using Pearson's method for normally distributed variables and Spearman's method for variables that could not be transformed to approach normality. Regressions and correlations were performed through the origin (Felsenstein, 1985).

Results of simple linear regressions

All three estimates of geographical range size were highly correlated (Tables S1.3 & S1.4 in Appendix 1.3). Furthermore, niche breadth was positively correlated with connectivity, and negatively correlated with niche position and occupancy of suitable habitat with raw species data (Table S1.3 in Appendix 1.3) and with independent contrasts (Table S1.4 in Appendix 1.3). Niche position was negatively related to connectivity with raw species data but not with independent contrasts, and unrelated to occupancy of suitable habitat (Tables S1.3 & S1.4 in Appendix 1.3). Amount of suitable habitat was positively related to connectivity and negatively related to occupancy of suitable habitat (Tables S1.3 & S1.4 in Appendix 1.3). Consistent with the hypothesized relationships in Fig. 2.2a, niche breadth was positively related to the number of climatically suitable pixels across the study region and range size, niche position was negatively related to the number of climatically suitable pixels across the study region and range size (Tables S1.5 & S1.6 in Appendix 1.3; Figs S1.5a–d & S1.6a–d in Appendix 1.1), and the number of climatically suitable pixels was positively related to range size (Tables S1.5 & S1.6 in Appendix 1.3; Figs S1.5e & S1.6e in Appendix 1.1). Although three outlier species that are endemic to Mexico may appear to have a low niche position because of geographical marginality in relation to the study region, which thus may have driven this relationship, niche position was even more strongly negatively related to amount of suitable habitat and range size when we

removed these three outliers (Table S1.5 in Appendix 1.3; Fig. S1.5b, d in Appendix 1.1). With raw species data, habitat connectivity was negatively related to occupancy of suitable habitat within species' ranges and unrelated to occupancy of suitable habitat once outliers were removed (Table S1.5 in Appendix 1.3; Fig. S1.5f in Appendix 1.1), and with independent contrasts habitat connectivity was unrelated to occupancy (Table S1.6 in Appendix 1.3; Fig. S1.6f in Appendix 1.1), providing no support for the prediction of a positive relationship. Occupancy of suitable habitat was not related to range size (Tables S1.5 & S1.6 in Appendix 1.3; Figs S1.5g & S1.6g in Appendix 1.1), and habitat connectivity was positively related to range size (Tables S1.5 & S1.6 in Appendix 1.3; Figs S1.5h & S1.6h in Appendix 1.1).

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APPENDIX 1.3

Table S1.1 Data sources for western North American *Mimulus* species.

Accessed through the California Consortium of Herbaria, 30 June 2011
California Academy of Sciences
California Department of Food and Agriculture
California State University, Chico
Humboldt State University
New York Botanical Garden
Rancho Santa Ana Botanic Garden and Pomona College combined herbaria
San Diego Natural History Museum
San Diego State University
San Jose State University
Santa Barbara Botanic Garden
UC Berkeley
UC Davis
UC Irvine
UC Riverside
UC Santa Barbara
UC Santa Cruz
Accessed through the Consortium of Pacific Northwest Herbaria, 13 May 2010
B. A. Bennett Herbarium, Yukon Government
Oregon State University
Royal British Columbia Museum
The New York Botanical Garden
University of Alaska, Fairbanks - Museum of the North
University of Washington
Accessed through the Southwest Environmental Information Network, 7 April 2010
Arizona State University Vascular Plant Herbarium
Cochise County Herbarium
Colorado State University Herbarium
Comisión Nacional para el Conocimiento y Uso de la Biodiversidad
Deaver Herbarium
Desert Botanical Garden Herbarium Collection
ENMU Natural History Collection Herbarium
Grand Canyon National Park
Herbario de la Universidad de Sonora
Intermountain Herbarium
Madrean Archipelago Biodiversity Assessment Observations
Navajo Nation Herbarium

New Mexico State University Herbarium
New York Botanical Garden
University of Arizona Herbarium
University of California, Riverside Plant Herbarium
University of Colorado Museum Herbarium
University of New Mexico Herbarium
US Forest Service Southwestern Region - TEUI Herbarium
Other
Angert lab field collections, 1998–2011
Missouri Botanical Garden, 22 November 2006
Pullen Herbarium, 17 November 2006
Rocky Mountain Herbarium, 4 February 2010

Table S1.2 Area under the receiver operating characteristic curve (AUC) values from MAXENT models trained with all occurrence points, and mean AUC ± 1 standard error (SE) from MAXENT models using cross-validation with two replicates for species with $n < 10$, and 10 replicates for species with $n \geq 10$.

	Models trained with all occurrence points		Cross-validation models with replicates	
Mimulus species	*n*	Training AUC full	Mean training AUC ± 1 SE	Mean test AUC ± 1 SE
M. alsinoides	98	0.985	0.985 ± 0	0.981 ± 0.001
M. androsaceus	37	0.995	0.995 ± 0	0.994 ± 0.001
M. angustatus	30	0.997	0.998 ± 0	0.997 ± 0.001
M. aurantiacus	551	0.968	0.97 ± 0	0.968 ± 0.001
M. bicolor	72	0.994	0.994 ± 0	0.994 ± 0.001
M. bigelovii	206	0.976	0.978 ± 0	0.972 ± 0.002
M. bolanderi	76	0.993	0.993 ± 0	0.992 ± 0.001
M. breviflorus	49	0.974	0.974 ± 0.001	0.965 ± 0.008
M. brevipes	164	0.990	0.99 ± 0	0.988 ± 0.001
M. breweri	256	0.973	0.975 ± 0	0.969 ± 0.003
M. calciphilus	3	0.990	0.748 ± 0.248	0.729 ± 0.229
M. cardinalis	333	0.972	0.973 ± 0	0.969 ± 0.001
M. clevelandii	23	0.997	0.997 ± 0	0.996 ± 0.001
M. clivicola	5	0.976	0.976 ± 0.003	0.976 ± 0.004
M. congdonii	40	0.993	0.993 ± 0	0.992 ± 0.001
M. constrictus	40	0.997	0.998 ± 0	0.997 ± 0
M. cordatus	7	0.909	0.933 ± 0.036	0.894 ± 0.045
M. cusickii	56	0.981	0.981 ± 0	0.972 ± 0.006
M. dentatus	54	0.993	0.993 ± 0	0.992 ± 0.001
M. dentilobus	43	0.951	0.949 ± 0.001	0.914 ± 0.016
M. douglasii	85	0.991	0.990 ± 0	0.989 ± 0.001
M. dudleyi	9	0.994	0.994 ± 0.003	0.993 ± 0.001
M. eastwoodiae	21	0.993	0.993 ± 0	0.991 ± 0.002
M. exiguus	8	0.961	0.959 ± 0.015	0.944 ± 0.007
M. filicaulis	6	0.997	0.997 ± 0.001	0.997 ± 0.001
M. floribundus	441	0.908	0.910 ± 0.001	0.882 ± 0.01
M. fremontii	128	0.986	0.988 ± 0	0.984 ± 0.004
M. gemmiparus	5	0.990	0.993 ± 0.006	0.982 ± 0.008
M. glaucescens	28	0.998	0.998 ± 0	0.998 ± 0.001
M. gracilipes	5	0.995	0.993 ± 0.002	0.991 ± 0.002
M. guttatus	1993	0.818	0.825 ± 0	0.814 ± 0.005
M. inconspicuus	40	0.996	0.996 ± 0	0.996 ± 0.001
M. johnstonii	22	0.997	0.997 ± 0	0.996 ± 0.002
M. jungermannioides	5	0.988	0.986 ± 0.007	0.985 ± 0.008

M. kelloggii	76	0.994	0.994 ± 0	0.993 ± 0.001
M. laciniatus	32	0.995	0.996 ± 0	0.994 ± 0.002
M. latidens	43	0.989	0.989 ± 0	0.986 ± 0.003
M. layneae	145	0.989	0.989 ± 0	0.988 ± 0.001
M. leptaleus	32	0.995	0.996 ± 0	0.993 ± 0.003
M. lewisii_north	65	0.995	0.967 ± 0	0.959 ± 0.003
M. lewisii_Sierra	208	0.966	0.995 ± 0	0.995 ± 0.001
M. mohavensis	17	0.998	0.998 ± 0	0.987 ± 0.011
M. montioides	43	0.990	0.991 ± 0	0.988 ± 0.004
M. moschatus	439	0.956	0.957 ± 0	0.951 ± 0.002
M. nanus	371	0.968	0.970 ± 0	0.967 ± 0.002
M. norrisii	5	0.992	0.994 ± 0.005	0.990 ± 0.002
M. nudatus	13	0.999	0.999 ± 0	0.999 ± 0
M. pallens	21	0.983	0.982 ± 0.001	0.950 ± 0.026
M. palmeri	71	0.992	0.993 ± 0	0.991 ± 0.001
M. parishii	48	0.993	0.994 ± 0	0.992 ± 0.001
M. parryi	18	0.988	0.988 ± 0	0.985 ± 0.005
M. patulus	8	0.912	0.971 ± 0.01	0.776 ± 0.034
M. pictus	13	0.997	0.998 ± 0	0.997 ± 0.001
M. pilosus	334	0.965	0.965 ± 0	0.958 ± 0.002
M. primuloides	272	0.973	0.974 ± 0	0.968 ± 0.005
M. pulchellus	12	0.998	0.998 ± 0	0.998 ± 0.001
M. pulsiferae	81	0.991	0.990 ± 0	0.989 ± 0.001
M. purpureus	13	0.980	0.983 ± 0.001	0.960 ± 0.021
M. pygmaeus	16	0.997	0.995 ± 0	0.994 ± 0.001
M. rattanii	20	0.997	0.998 ± 0	0.997 ± 0.001
M. rubellus	232	0.936	0.937 ± 0.001	0.908 ± 0.008
M. rupicola	5	0.996	0.998 ± 0	0.993 ± 0.001
M. shevockii	4	0.992	0.992 ± 0.001	0.987 ± 0.002
M. suksdorfii	148	0.962	0.962 ± 0	0.948 ± 0.005
M. tilingii	220	0.969	0.970 ± 0	0.963 ± 0.004
M. torreyi	115	0.993	0.993 ± 0	0.993 ± 0
M. tricolor	106	0.990	0.990 ± 0	0.987 ± 0.002
M. verbenaceus	54	0.930	0.932 ± 0.001	0.889 ± 0.016
M. viscidus	27	0.997	0.997 ± 0	0.996 ± 0.001
M. washingtonensis	14	0.964	0.964 ± 0.003	0.937 ± 0.02
M. whitneyi	30	0.998	0.998 ± 0	0.997 ± 0
M. wiensii	4	0.992	0.994 ± 0.004	0.968 ± 0.01

Table S1.3 Bivariate correlations between pairs of explanatory variables and between pairs of response variables (geographical range size measured as *EOO*, extent of occurrence; *LOE*, longitudinal extent; and *LAE*, latitudinal extent; *NB*, niche breadth; *NP*, niche position; *CONN*, connectivity; NSP_{total}, total number of climatically suitable pixels; NSP_{occ}/NSP_{total}, proportion of climatically suitable pixels that are occupied). When possible, variables were transformed to meet assumptions of normality. All correlation coefficients were Pearson's except when denoted ^{s}, in which case Spearman's correlation coefficient is reported because at least one variable was not normally distributed. *Significant at $P < 0.05$.

Variable 1	Variable 2	Correlation coefficient
$\text{Log}_{10}(EOO)$	$\text{Log}_{10}(LOE)$	0.953*
$\text{Log}_{10}(EOO)$	$LAE^{0.25}$	0.952*
$\text{Log}_{10}(LOE)$	$LAE^{0.25}$	0.902*
$NB^{0.25}$	NP	$-0.543^{*}{}_{s}$
$NB^{0.25}$	$CONN$	0.624^{*s}
$NB^{0.25}$	$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	–0.518*
NP	$CONN$	-0.645^{*s}
NP	$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	0.181^{s}
$CONN$	$\text{Log}_{10}(NSP_{total})$	0.769^{*s}
$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	$\text{Log}_{10}(NSP_{total})$	–0.529*

Table S1.4 Bivariate Pearson's correlation coefficients between pairs of explanatory variables and between pairs of response variables in the form of phylogenetically independent contrasts. Correlation coefficients were computed through the origin. *Significant at $P < 0.05$. Abbreviations as in Table S2.3.

Variable 1	Variable 2	Correlation coefficient
$\text{Log}_{10}(EOO)$	$\text{Log}_{10}(LOE)$	0.954*
$\text{Log}_{10}(EOO)$	$LAE^{0.25}$	0.950*
$\text{Log}_{10}(LOE)$	$LAE^{0.25}$	0.863*
$NB^{0.25}$	NP	–0.481*
$NB^{0.25}$	$CONN$	0.334*
$NB^{0.25}$	$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	–0.510*
NP	$CONN$	–0.170
NP	$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	0.081
$CONN$	$\text{Log}_{10}(NSP_{total})$	0.474*
$\text{Log}_{10}(NSP_{occ}/NSP_{total})$	$\text{Log}_{10}(NSP_{total})$	–0.468*

Table S1.5 Summary of simple linear regression analyses of geographical range size metrics regressed on niche properties and colonization ability variables. Variables were transformed to meet normality assumptions and improve model fit. Abbreviations as in Table S2.3.

Response	Predictor	Prediction	*B*	R^2	*P*
Niche properties					
Log_{10} (NSP_{total})	$NB^{0.25}$	+	2.689	0.760	< 0.001
Log_{10} (NSP_{total})	*NP* (all species)	–	–0.096	0.169	< 0.001
Log_{10} (NSP_{total})	*NP* (outliers removed)	–	–0.384	0.607	< 0.001
Log_{10} (*EOO*)	$NB^{0.25}$	+	2.893	0.337	< 0.001
Log_{10} (*EOO*)	*NP* (all species)	–	–0.161	0.181	< 0.001
Log_{10} (*EOO*)	*NP* (outliers removed)	–	–0.475	0.381	< 0.001
Log_{10} (*EOO*)	NSP_{total}	+	1.130	0.489	< 0.001
Colonization ability					
Log_{10} ($NSP_{\text{occ}}/NSP_{\text{total}}$)	*CONN* (all species)	+	–0.117	0.111	0.004
Log_{10} ($NSP_{\text{occ}}/NSP_{\text{total}}$)	*CONN* (outliers removed)	+	–0.135	0.029	0.161
Log_{10} (*EOO*)	Log_{10} ($NSP_{\text{occ}}/NSP_{\text{total}}$)	+	0.221	0.015	0.305
Log_{10} (*EOO*)	*CONN* (all species)	+	0.261	0.169	< 0.001
Log_{10} (*EOO*)	*CONN* (outliers removed)	+	0.950	0.413	< 0.001

Table S1.6 Summary of simple linear regression analyses of geographical range size metrics regressed on niche properties and colonization ability variables for phylogenetically independent contrasts. Abbreviations as in Table S2.3.

Response	Predictor	Prediction	*B*	R^2	*P*
Niche properties					
$\text{Log}_{10}(NSP_{\text{total}})$	$NB^{0.25}$	+	2.797	0.844	< 0.001
$\text{Log}_{10}(NSP_{\text{total}})$	*NP*	–	–0.249	0.421	< 0.001
$\text{Log}_{10}(EOO)$	$NB^{0.25}$	+	3.351	0.477	< 0.001
$\text{Log}_{10}(EOO)$	*NP*	–	–0.393	0.410	< 0.001
$\text{Log}_{10}(EOO)$	$\text{Log}_{10}(NSP_{\text{total}})$	+	1.263	0.628	< 0.001
Colonization ability					
$\text{Log}_{10}(NSP_{\text{occ}}/NSP_{\text{total}})$	*CONN*	+	–0.090	0.027	0.179
$\text{Log}_{10}(EOO)$	$\text{Log}_{10}(NSP_{\text{occ}}/NSP_{\text{total}})$	+	0.081	0.002	0.706
$\text{Log}_{10}(EOO)$	*CONN*	+	0.414	0.189	< 0.001

APPENDIX 2

Table S2.1 Akaike Information Criterion (AIC) values for three functions relating relative growth rate (*RGR*) to temperature (*T*). For *M. cardinalis*, *M. parishii*, *M. verbenaceus*, *M. eastwoodiae*, *M. floribundus*, and *M. norrisii*, we fitted thermal performance curves to *RGR* in leaf number, and for *M. bicolor*, *M. filicaulis*, *M. guttatus, and M. laciniatus*, we fitted curves to *RGR* in stem length.

Quadratic: $RGR = a + bT + cT^2$

Gaussian: $RGR = ae^{-0.5(\frac{[T-b]}{c})^2}$

Kumaraswamy: $RGR = ab\left(\frac{T-c}{d-c}\right)^{a-1}\left(1-\left(\frac{T-c}{d-c}\right)^{a}\right)^{b-1} * e$

Species	Quadratic	Gaussian	Kumaraswamy
M. cardinalis	-919.38	-928.02	-955.35
M. parishii	-1601.69	-1616.06	-1635.48
M. verbenaceus	-1038.21	-1032.07	-1038.99
M. eastwoodiae	-1748.65	-1750.47	-1754.65
M. floribundus	-419.22	-409.82	-421.74
M. norrisii	-585.92	-580.91	-582.86
M. bicolor	-311.87	-322.86	-320.35
M. filicaulis	-289.60	-298.90	-298.08
M. guttatus	-0.77	-18.29	-15.12
M. laciniatus	-101.19	-131.73	-132.90

Table S2.2 Widespread and restricted species pairs (denoted by species with the same letter subscript) with thermal performance curve parameter estimates and standard errors in parentheses based on the function with lowest AIC for each species pair (Table S3.1). Kumaraswamy functions were used for *M. cardinalis*, *M. parishii*, *M. verbenaceus*, and *M. eastwoodiae*, quadratic functions were used for *M. floribundus* and *M. norrisii*, and Gaussian functions were used for *M. bicolor*, *M. filicaulis*, *M. guttatus*, and *M. laciniatus*.

Species	*a*	*b*	*c*	*d*	*e*
M. cardinalis[a]	5.0297 (9.6818)	1.6020 (0.5649)	0.0169 (0.0265)	-41.7866 (140.0574)	42.8386 (0.8120)
M. parishii[a]	2.8651 (1.3166)	1.7845 (0.4111)	0.0445 (0.0131)	-6.7593 (15.7290)	43.4152 (1.0063)
M. verbenaceus[b]	1.6747 (1.1715)	1.5477 (0.3846)	0.0216 (0.0076)	-2.1777 (25.9027)	42.5 (0.0006)
M. eastwoodiae[b]	5.0550 (6.2427)	6.6078 (12.3484)	0.0160 (0.0146)	-23.5800 (56.5170)	48.6824 (16.4937)
M. floribundus[c]	-0.0003 (0.0000)	0.0159 (0.0019)	-0.0713 (0.0213)	NA	NA
M. norrisii[c]	-0.0002 (0.0000)	0.0112 (0.0011)	-0.0604 (0.0123)	NA	NA
M. bicolor[d]	0.2252 (0.0146)	27.3343 (0.6395)	8.5723 (0.656)	NA	NA
M. filicaulis[d]	0.1277 (0.0111)	28.4099 (0.8049)	8.1058 (0.8224)	NA	NA
M. guttatus[e]	0.4420 (0.0539)	32.2227 (0.8492)	6.0432 (0.8693)	NA	NA
M. laciniatus[e]	0.3501 (0.0286)	30.3411 (0.5184)	5.4491 (0.533)	NA	NA